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HEPATITIS C

GENERALIZATION OF TRIALS TO CLINICAL PRACTICE

FLOOR BERDEN

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Hepatitis C: Generalization of Trials to Clinical Practice

Floor Berden

The work presented in this thesis was carried out at the department of Gastroenterology and Hepatology of Radboudumc, within the Radboud Institute for Health Sciences.

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General introduction

1

Hepatitis C

The hepatitis C virus (HCV) is a hepatotropic RNA virus causing infectious hepatitis. The disease, formerly called non-A-non-B-hepatitis, was known for centuries, but was discovered only decades ago in 1989 when researchers isolated HCV RNA from chimpanzee plasma.[1, 2] Worldwide about 130- 150 million patients are infected with HCV, in the Netherlands the number of patients is estimated to be around 19,200.[3, 4] There are seven different HCV genotypes identified, and each genotype has an unique geographic distribution. Genotype 1 is most prevalent (50%), followed by genotype 3 (30%), both globally and in the Netherlands.[5, 6] Hepatitis C is a blood-borne disease and highest prevalence of disease is found in certain risk groups: people who inject drugs (PWID), haemophilia patients who received blood products prior to screening of transfusions (1992), HIV-infected patients, and men who have sex with men (MSM).[7] Once a person is HCV infected, there is 20-25% chance of spontaneous viral clearance, but the majority of patients will become chronically infected.[8] Often these patients are diagnosed accidentally or through screening activities, because an acute and chronic HCV infection causes no or only mild symptoms, such as malaise, fatigue and exceptionally jaundice.[9] Patients with chronic hepatitis C can develop fibrosis (scarring of the liver) over the years due to constant liver inflammation, which can progress to cirrhosis. This development is undesirable because cirrhosis can decompensate with ensuing complications such as variceal bleeding, hepatic encephalopathy and ascites. In addition cirrhotic patients have a 1-4% annually risk of developing hepatocellular carcinoma (Figure 1).[10] Furthermore HCV may cause extrahepatic manifestations, such as mixed cryoglobulinemia, B-cell non-Hodgkin lymphoma, diabetes, and atherosclerosis resulting in morbidity and even mortality.[11]

Figure 1. Natural course of disease of hepatitis C virus infection.

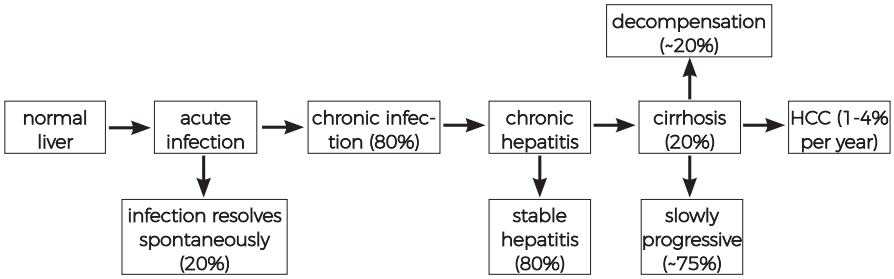


Figure adapted from Lauer et al. NEJM 2001; HCC = hepatocellular carcinoma

Treatment of hepatitis C

The primary goal of HCV therapy is to cure the patient by eradicating HCV to prevent cirrhosis and spread of the virus. Both clinical trials and clinical practice use sustained

virological response (SVR) as a surrogate biomarker for cure. SVR is an objective parameter and defined as undetectable HCV RNA 12 or 24 weeks after cessation of treatment.[12] Achievement of SVR has been associated with reduced liver-related and all-cause mortality.[13]

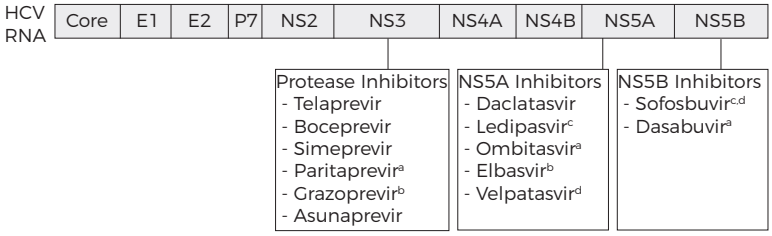
For years, therapy consisted of (peg-)interferon (pegIFN) and ribavirin (RBV). PegIFN was injected subcutaneously once weekly and depending on genotype given for 24 or 48 weeks. The disadvantage of pegIFN was the moderate chance for SVR (66-80% in genotype 2 and 3, 45% in genotype 1 and 4) and relatively high risk of (severe) toxicity. The most common adverse events are: flu-like symptoms, anemia, neutropenia, thrombocytopenia, depression, and fatigue. The addition of RBV, an oral guanosine analog, has improved the SVR rate, but also intensified toxicity, mainly by inducing abdominal complaints, hemolytic anemia and rash.[14]

The advent of direct acting antivirals (DAAs) in 2012 changed the therapeutic outlook enormously. All-oral interferon-free treatments were available and improved effectiveness, safety and quality of life of HCV patients.[15] Currently there are 3 classes of DAAs developed which target the virus directly at three different proteins of the virus. Each class contains several DAAs. Figure 2 shows the HCV RNA structure of the virus, with the targets of the DAAs and the various DAAs in each class:[14, 16]

- **NS3/4A protease inhibitors (name: -previr)**
  NS3/4A protease inhibitors target the NS3/4A serine protease which is responsible for production of new virions. First-generation protease inhibitors, telaprevir and boceprevir, were approved in 2011 and were added to pegIFN and RBV. These agents improved efficacy significantly in clinical trials, however also caused severe toxicity, such as anemia, infections, and rash. In 2013 simeprevir has been approved, a second generation protease inhibitor with a more favorable safety profile. There are two other agents approved in this class: paritaprevir with ritonavir and grazoprevir, both are combined with other DAAs in one pill. Protease inhibitors are mainly effective against genotype 1 and 4.
- **NS5A inhibitors (name: -asvir)**
  NS5A inhibitors target the non-structural 5A protein and inhibit viral assembly and replication. Currently, there are four NS5A inhibitors approved by regulatory authorities: daclatasvir, ledipasvir, velpatasvir, and ombitasvir. All but the latter are combined with sofosbuvir.
- **NS5B polymerase inhibitors (name: -buvir)**
  The NS5B polymerase inhibitors are involved in the HCV RNA replicative machinery. Currently, sofosbuvir is the most important NS5B polymerase inhibitor because it can be combined with both protease inhibitors as NS5A inhibitors, it possesses a high barrier for resistance and a high effectiveness against all genotypes. Another NS5B inhibitor is dasabuvir, this agent is developed together with paritaprevir/ritonavir and ombitasvir.

The latest generation DAAs have a high efficacy, on average >90% in genotype 1, 2, and 4. Genotype 3 remains somewhat more difficult to cure.[17] Further the DAAs have low toxicity, compared to pegIFN and RBV. The main side effects of DAAs are fatigue, nausea, headache and insomnia. In case ribavirin is added, patients may develop (hemolytic) anemia, diarrhea, and loss of appetite.[18] Treatment discontinuation due to adverse events occurs around 1-2% of treatments. There is a comparable low risk of serious adverse events in all DAAs.[19, 20] A remaining issue in current clinical practice is the risk of drug-drug interactions of DAAs with concomitant medication that the HCV patient is using for other conditions. DAAs are substrate and inhibitor of several cytochrome P450 (CYP) enzymes and drug transporters which increases the risk of interactions. This can result in loss of efficacy or increased toxicity of both the DAA and the co-medication.[21]

Figure 2. Classes of approved direct acting antiviral (DAA) agents which target the virus.

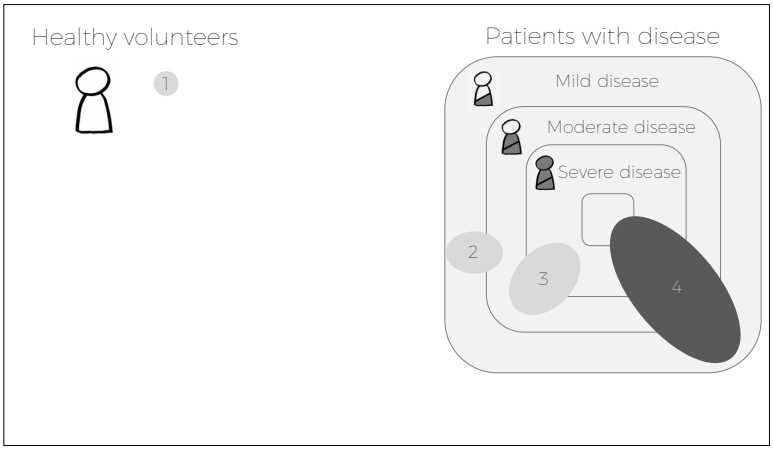


DAAs target the non-structural (NS) proteins of the HCV RNA in order to eradicate the virus. There are 3 classes: 1) Protease inhibitors target the NS3/4A serine protease, 2) NS5A inhibitors target the NS5A protein and 3) NS5B inhibitors target the NS5B polymerase inhibitor. Several DAAs are developed within the classes. Agents marked by a, b, c, d are all developed as one combination.

Approval and registration of new drugs

Research into new drugs is mainly done by pharmaceutical companies and pass over 2 stages (pre-clinical and clinical). Pre-clinical studies are done to assess biological activity. The clinical studies are executed in humans in 3 phases (Figure 3). Phase 1 trials aim to determine drug dose and side effects and are performed in healthy volunteers. Phase 2 trials aim to assess the most effective dose, while monitoring adverse events in patients. Phase 3 trials, ideally randomized clinical trials (RCTs), are designed to confirm efficacy in various populations of patients (representative for the whole disease spectrum within clinical practice). These phase 3 trials are often used for registration by regulatory authorities such as U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA). FDA or EMA then checks if the drug is safe and effective, and if benefits outweigh the risks.[22, 23] After approval, phase 4 (post-marketing) surveillance can be conducted and some countries will assess the cost-effectiveness of the drug for reimbursement. In the Netherlands this is done by ZIN (ZorgInstituut Nederland), ZIN compares the therapeutic value of the new drug to standard of care, and evaluates the budget impact and cost-effectiveness of the new drug.[24]

Figure 3. Schematic visualization of population included in clinical trials



This figure shows which part of the population is included in different phases of drug development: 1 (phase 1 trial), 2 (phase 2 trial), 3 (phase 3 trial), and 4 (phase 4 data). The size represents the size of the trial. Light grey represents trials before registration and dark grey after registration; it is clear that not the whole disease spectrum is included in phase 1-2-3 research.

Generalizability of registration trials

Ideally, phase 3 registration trials include patients that resemble the clinical practice population, however designing the perfect trial with both a high internal and high external validity is difficult. Generalizability or external validity of registration trials appeared to be limited in some disease areas (e.g. psoriasis, rheumatic arthritis, inflammatory bowel disease) which hampers direct use of the data in clinical practice.[25, 26] Often, trials use strict eligibility criteria in order to increase internal validity and to select easy-to-treat patients with the highest chance of efficacy and limited risk of toxicity. While in clinical practice the difficult-to-treat patients receive treatment.[27] This can be called a development paradox which can result in limited generalizability of outcomes of registration trials. Still, physicians, guideline developers and policy makers do need to choose or recommend specific regimens for a variety of patients.

Some essential components in the translation of registration trials to clinical practice are: generalizability of trials and use of an adequate comparator. There are some ways to assess generalizability of trials: a) to examine eligibility criteria and assess whether the trial population was sufficiently representative, b) to use data from observational cohorts, when no direct evidence is available for the population, and c) to use relative effects found in RCTs and apply this to the population to estimate the absolute effect.[28] The latter can be done with statistical techniques such as network meta-analysis and Bayesian statistics. Apart from generalizability, the comparative arm in studies is important for physicians and guideline developers. Ideally a standard of care option should be included in the trials to adequately assess comparative effectiveness.

Concerns about registration trials in hepatitis C

In HCV the development paradox was clear in the case of first-generation protease inhibitors: registration trials showed improved efficacy with tolerable toxicity in easy-to-treat patients, while the first real world cohorts demonstrated high risk of serious adverse events and even mortality in patients with cirrhosis.[29, 30] Here, the registration trials included standard of care (PegIFN and RBV) as the comparative arm. For trials with newer DAAs there were two reasons which made inclusion of the standard of care complex: 1) the field developed at such a rapid pace that the standard of care at initiation of the trial was different from the time of introduction to the market; 2) the DAAs are developed by many pharmaceutical companies, and head-to-head trials are not part of their portfolio. The design of many registration trials in hepatitis C appears suboptimal, for example the ALLY-3 trial was a single arm trial including 152 easy-to-treat HCV genotype 3 patients with only 21% cirrhotics, who received sofosbuvir with daclatasvir for 12 weeks.[31] This trial led to the FDA approval of sofosbuvir with daclatasvir for 12 weeks in all HCV genotype 3 patients and illustrates the development paradox.[32]

Outline and aims of this thesis

The therapeutic landscape in HCV treatment has changed and is still changing. Many registration trials that have been executed used strict eligibility criteria, resulting in a gap between trials and clinical practice. The main aim of this thesis was to improve translation of results from clinical trials with direct acting antivirals in chronic hepatitis C to clinical practice. We will study two steps of translation of trials in hepatitis C: generalizability of trials and interpretation of evidence in clinical practice.

Generalizability of trials to clinical practice

In **chapter 2** we answered the research question: What is the effectiveness and safety of real world patients who are eligible vs. ineligible for registration trials? We hypothesized that a high proportion of HCV patients treated in clinical practice would not be eligible for trials and that characteristics for ineligibility were risk factors for treatment failure. To investigate this hypothesis we retrospectively collected data in a nationwide registry of all patients treated with first-generation protease inhibitors combined with pegIFN and RBV. In addition we aimed to identify the criteria that impact trial eligibility and to assess the risk of these criteria on effectiveness and safety.
Based on the data of this registry we performed two studies on specific adverse events (infections and bleeding episodes) in clinical practice. It is thought that neutropenia and thrombocytopenia, two important side effects of pegIFN, increase the risk of infections and bleeding episodes respectively. In that case, strict dose reduction of pegIFN is recommended, which may lead to reduced effectiveness.[33, 34]

The rational to perform studies with first-generation protease inhibitors combined with pegIFN and RBV were clear: (i) there is data that protease inhibitors increase the risk of severe infection[29, 35], and (ii) a meta-analysis demonstrated more thrombocytopenia with protease inhibitors which might lead to more bleeding episodes.[36] In **chapter 3** we answered the research question: What is the prevalence and what are the risk factors for infections during triple therapy with first-generation protease inhibitors? We hypothesized that these drugs result in a high risk of infections, but without a relation to neutropenia. In **chapter 4** we answered the research question: What are the prevalence and risk factors for bleeding episodes during triple therapy with first-generation protease inhibitors? Here the hypothesis was that protease inhibitors are associated with thrombocytopenia but not with bleeding episodes.

Over time new DAAs were trialed and approved by regulatory authorities (FDA and EMA). The new generation DAAs are administered without pegIFN, which contributed to the better safety profile of these drugs. We hypothesized that this led to less strict eligibility criteria in the registration trials of these new DAA regimens, probably resulting in a better generalizability. In **chapter 5** we tried to find an answer to the research question: does the eligibility rate of clinical practice patients for DAA trials improve over time? Here we focused on the most important eligibility criteria identified in **chapter 2.** We studied these criteria in all registration trials of new DAAs and collected cross-sectional data of all HCV infected patients in two hospitals (tertiary and regional center). We determined the eligibility rate of this cohort on the selected criteria.

As toxicity decreased and efficacy increased with the new generation DAAs not all issues in clinical practice were solved. As shown in **chapter 2** prohibited concomitant medication was one of the most frequent reasons for exclusion from a trial. The rational here is that DAAs inhibit/induce and can be substrates of drug-metabolizing enzymes and drug transporters, so DAAs have the potential to cause drug-drug interactions.[21] In **chapter 6** we answered the following research question: What concomitant medication do HCV patients use, and what is the predicted risk on drug-drug interactions with DAAs for hepatitis C? We hypothesized that use of concomitant medication is diverse and that many patients use concomitant medication with risk of interaction. For this study we used baseline data of the registry of patients treated with first-generation protease inhibitors.

Interpretation of evidence in clinical practice

It is difficult to interpret evidence from trials into clinical practice in the presence of a development paradox and lack of head-to-head trials. A way to deal with this lack of head-to-head trials is by using the technique of network meta-analysis (NMA) with Bayesian statistics. A NMA is able to compare more than 2 treatments. By combining direct and indirect evidence using Bayesian statistics NMA is able to estimate outcomes

such as mean SVR rate per DAA regimen.[37, 38] In **chapter 7** we performed a systematic review and NMA to answer the research question: What is the most effective DAA regimen for HCV genotype 3 patients and what is the role of ribavirin here? Genotype 3 turned out to be the most difficult genotype to cure during the drug development phase and head-to-head trials of DAA regimens were scarce, therefore the technique of NMA was suitable to indirectly compare efficacy of various DAA regimens in this genotype.

The ultimate translation of evidence is done in guideline development. Guidelines provide an overview and take multiple factors of both trials and practice into account, such as efficacy, safety, settings, costs/reimbursement, and availability. With this information guideline developers attempt to steer physicians in clinical practice to improve and standardize care. The need for a guideline for the treatment of hepatitis C in the Netherlands was high, because the last guideline dated from 2013, included only first-generation protease inhibitors and was already outdated soon after publication.[33] However, the latter was the reason why a formal guideline was not suitable for hepatitis C in the past years. In **chapter 8** we developed a guidance for the treatment of hepatitis C which served as a dynamic document. The first version was published and based on a systematic review of sofosbuvir combined with (pegIFN and) RBV. We formulated recommendations based on the GRADE method.[39] Later we established a guidance committee of five associations: NVMDL (Netherlands Association of Hepato-gastroenterologists), NVH (Netherlands Association of Hepatology), NIV (Netherlands Association of Internal Medicine), NVHB (Dutch Association of HIV- treating physicians), NVZA (Netherlands Association of Hospital Pharmacists) and produced an online guidance which was updated every 3-6 months (www.hcvrichtsnoer.nl). This online guidance is based on international guidelines, a practical and suitable way to provide the most updated information to Dutch physicians in clinical practice.

Finally, in **chapter 9** we describe a general discussion on the generalization and interpretation of evidence from trials to clinical practice in hepatitis C, including alternatives for this problem.

Abbreviations

CYP	CYtochrome P450
DAAs	Direct-Acting Antivirals
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
MSM	Men who have Sex with Men
NIV	Netherlands Association of Internal Medicine
NMA	Network Meta-Analysis
NS	Non-Structural
NVH	Netherlands Association of Hepatology
NVHB	Dutch Association of HIV-treating physicians
NVMDL	Netherlands Association of Hepato-gastroenterologists
NVZA	Netherlands Association of Hospital Pharmacists
pegIFN	peg-interferon
PWID	People Who Inject Drugs
RBV	Ribavirin
RCT	Randomized Clinical Trial
SVR	Sustained Virological Response
ZIN	ZorgInstituut Nederland

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2

Limited generalizability of registration trials in hepatitis C: a nationwide cohort study

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Abstract

Background

Approval of drugs in chronic hepatitis C is supported by registration trials. These trials might have limited generalizability through use of strict eligibility criteria. We compared effectiveness and safety of real world hepatitis C patients eligible and ineligible for registration trials.

Methods

We performed a nationwide, multicenter, retrospective cohort study of chronic hepatitis C patients treated in real world. We applied a combined set of inclusion and exclusion criteria of registration trials to our cohort to determine eligibility. We compared effectiveness and safety in eligible vs. ineligible patients, and performed sensitivity analyses with strict criteria. Further, we used log binomial regression to assess relative risks of criteria on outcomes.

Results

In this cohort (n=467) 47% of patients would have been ineligible for registration trials. Main exclusion criteria were related to hepatic decompensation and co-morbidity (cardiac disease, anemia, malignancy and neutropenia), and were associated with an increased risk for serious adverse events (RR 1.45-2.31). Ineligible patients developed significantly more serious adverse events than eligible patients (27% vs. 11%, $p< 0.001$). Effectiveness was decreased if strict criteria were used.

Conclusions

Nearly half of real world hepatitis C patients would have been excluded from registration trials, and these patients are at increased risk to develop serious adverse events. Hepatic decompensation and co-morbidity were important exclusion criteria, and were related to toxicity. Therefore, new drugs should also be studied in these patients, to genuinely assess benefits and risk of therapy in the real world population.

Introduction

Regulatory approval of drugs and the development of guidelines are supported by evidence generated by registration trials. These trials aim for high internal validity through use of strict eligibility criteria, although this may jeopardize generalizability.[1,2] Some studies suggest that many real world patients would be excluded from registration trials and that drugs tested through these trials are less effective or less well tolerated in these patients.[3-5]

The treatment arsenal for chronic hepatitis C patients (CHC) has increased enormously with the introduction of Direct Acting Antivirals (DAAs). DAAs were approved by regulatory authorities for use in clinical practice, with evidence coming from registration trials having strict criteria.[6] Indeed, real world cohorts contain large number of treated CHC patients who would be excluded from registration trials.[7-10] A lack of generalizability is only an issue when ineligible patients have worse outcomes, but this is not known for CHC. We hypothesize that CHC patients ineligible for trials, but who are treated in clinical practice have characteristics that are risk factors for treatment failure and toxicity.

Therefore, we aim to compare effectiveness and safety in real world CHC patients who are eligible or ineligible for registration trials. Our secondary aim is to identify criteria that impact trial eligibility and assess the risk of these criteria on outcomes.

Materials and Methods

Population and design

We conducted a nationwide, multicenter, retrospective real world cohort study of CHC patients in the Netherlands. We chose genotype 1 patients treated between 2011 and 2015 with telaprevir or boceprevir with peg-interferon and ribavirin as an example cohort. We identified CHC patients using up-to-date local databases. Treatment indication, choice of therapy, drug dosing and duration were at the discretion of the physician, following national guidelines.[11] Patients co-infected with HIV or hepatitis B virus (HBV) were excluded.
Formal evaluation was waived by the institute review board committee on research involving human subjects Arnhem-Nijmegen given the retrospective character of our study. However, approval in participating centers was obtained according to local regulations. The study was conducted in accordance with good clinical practice guidelines and the code of conduct for medical research (www.federa.org). We obtained oral informed consent or collected data anonymously in accordance with the code of conduct for medical research. No identifying patient data was collected, and all patient data was anonymously entered in the database.

Identification of registration trials and general set of eligibility criteria

We identified registration trials of telaprevir and boceprevir in CHC patients through a systematic search (S1 Table). We extracted eligibility criteria from published protocols, and used the least stringent criteria of all studies to develop a general criteria set (Table 1). We applied the general set to our real world population to determine eligibility. If variables were missing, we assumed the patient would be eligible for that criterion.

Data acquisition and definitions

We extracted demographics, CHC characteristics, and laboratory values from the patient's medical records on a pre-designed case report form. Baseline variables were collected at the start of treatment not exceeding one year prior to treatment. Baseline concomitant medication was collected prior to possible medication switch for expected interactions. Data was collected until 24 weeks after cessation of treatment. We collected whether patients had a history of or current decompensated liver disease, this was defined as a history or signs of ascites, variceal bleed or hepatic encephalopathy. Effectiveness was defined as sustained virological response (SVR): undetectable hepatitis C virus RNA 12 or 24 weeks after cessation of treatment. Safety data included adverse events (AEs) and serious adverse events (SAEs). AEs were defined as any event that required 1) dose reduction of peg-interferon or ribavirin, 2) prescription of medication or 3) referral. We used the FDA definition for SAEs.[12] We categorized AEs and SAEs by common terminology criteria for adverse events (CTCAE version 4.0).[13] We recorded data anonymously in an Access database (Microsoft Access 2007).

Outcomes and analysis

The primary outcomes were SVR and (S)AE rates, which were compared between patients eligible and ineligible for registration trials. Furthermore, we identified criteria that affected eligibility and were associated with the outcomes. Analyses were performed on an intention to treat population, where telaprevir and boceprevir treated patients were pooled. To check validity of pooling, we compared baseline characteristics and treatment outcomes between telaprevir and boceprevir patients.[14]

SVR rates, and (S)AE rates were analyzed with chi-square (or Fisher exact if counts <5), and Mann-Whitney U test (median number of (S)AEs). For analyses on SVR, we separated patients into two groups based on expected similar effectiveness: 1) treatment-naive and relapse patients, and 2) patients with a prior non-response, viral breakthrough or early discontinuation[15]; for safety outcomes this distinction was not made. We used frequency counts to identify most important eligibility criteria. To study the association of criteria and outcomes, we performed log binomial regression (relative risk) or poisson regression.[16] To explore the validity of our generated set of the least stringent criteria from the protocols, we performed three sensitivity analyses: a) with most stringent

Table 1. Set of general eligibility criteria

Variable	Criterion
Inclusion	
Age	Subject ≥ 18 years
Hepatitis C virus (HCV) RNA	HCV RNA detectable
Weight	Weight between 40-125 kg
Hepatocellular Carcinoma (HCC)	Ultrasound with no signs of HCC
Exclusion	
Genotype HCV	HCV with > 1 subtype or genotype
Hemoglobin	Hemoglobin <12 g/dL (females) or <13 g/dL (males)
Neutrophil count	Absolute neutrophil count <1.2 x10^9/L
Platelet count	Platelet count <90 x10^9/L
Albumin	Serum albumin < 3.3 g/dL
Bilirubin	Total bilirubin > 1.8 xULN†
International Normalized Ratio (INR)	INR ≥ 1.5
Thyroid Stimulating Hormone (TSH)	TSH > 1.2 xULN or 0.8 xLLN†
Alanine aminotransferase (ALT)	ALT 10 xULN†
Aspartate aminotransferase (AST)	AST 10 xULN†
Contra-indication to peginterferon or ribavirin	
- Hemoglobinopathy	- Hemoglobinopathy present (thallassemia major, sickle-cell disease)
- Cardiac disease	- Significant cardiac disease present[a]
- Renal insufficiency	- Creatinine clearance ≤ 50 ml/min
Auto-immune disease	Presence of auto-immune disease[b]
Pulmonary disease	History of chronic pulmonary disease with impairment (COPD gold III or IV, interstitial lung disease, pulmonary fibrosis or sarcoidosis)
Current or history of decompensated liver disease	Current or history of ascites, encephalopathy or bleeding varices
Other liver disease	Presence of another liver disease
Malignancy	Active malignant disease or malignant disease in past 5 years (except basal cell carcinoma)
Pancreatitis	History of acute pancreatitis in past 5 years
Retinopathy	Presence of retinopathy
Seizure	Presence of a seizure disorder requiring medication
Transplantation	Patient with a history of an organ transplant
Psychiatric comorbidity	Presence of severe psychiatric disease[c]
Corticosteroids	Use of systemic corticosteroids
Hemophilia	Hemophilia present
Central nervous system (CNS) disorder	CNS disorder present[d]
Malabsorption	History of malabsorption disorder
Indwelling cathether	Subject with indwelling venous catheter
Comedication	Prohibited comedication listed in protocols

[a] Significant cardiac disease was defined as: current or history of unstable cardiac disease (angina, congestive heart failure, recent myocardial infarction, pulmonary hypertension, complex congenital heart disease, cardiomyopathy, and/or significant arrhythmia)

[b] Auto-immune disease was defined as: immunologically mediated disease (inflammatory bowel disease, celiac disease, rheumatoid arthritis, idiopathic thrombocytopenic purpura, systemic lupus erythematosus, auto-immune hemolytic anemia, scleroderma, sarcoidosis, severe psoriasis, or auto-immune hepatitis)

[c] Psychiatric comorbidity was defined as: severe depression or hospitalization for depression, schizophrenia, bipolar illness, severe anxiety or personality disorder, a period of disability or impairment due to a psychiatric disease within the past 5 years

[d] CNS disorder was defined as: CNS trauma requiring intubation, intracranial pressure monitoring, brain meningeal/skull surgery, or resulting in seizure, coma, neurologic deficits, abnormal brain imaging, cerebrospinal fluid leak, prior brain hemorrhage and/or intracranial aneurysms, or history of stroke or transient ischemic attack

† ULN = upper limit of normal; LLN = lower limit of normal

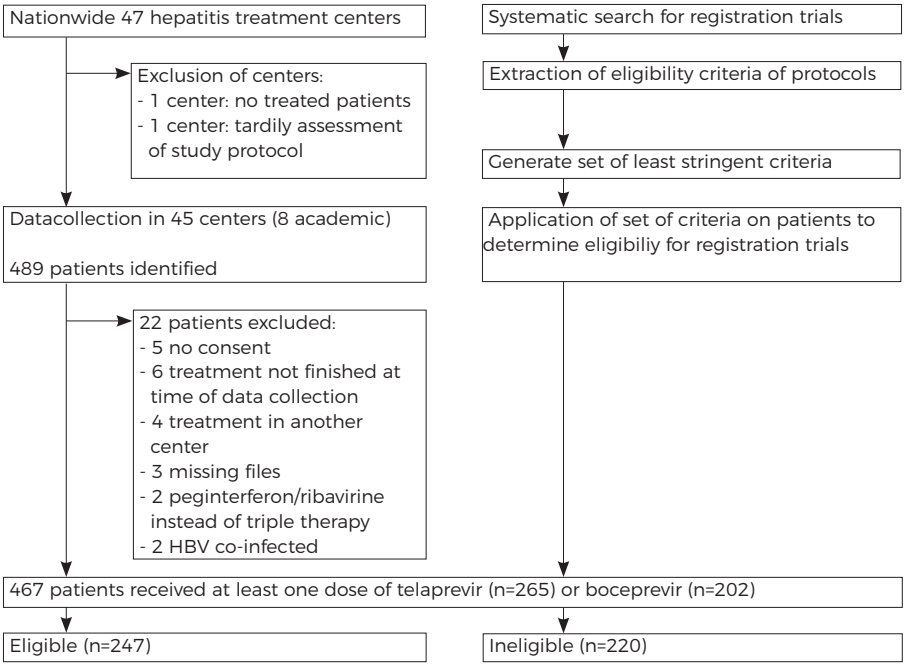
criteria (S2 Table), b) with strictest exclusion of co-morbidity, and c) with the most important factor for exclusion eliminated from the criteria set. All analyses were two-sided with a significance level of p <0.05, and performed in SPSS (IBM SPSS Statistics 20).

Results

Population

We identified 489 treated patients from 45 centers, and we excluded 22 patients (Figure 1). Centers treated a median of 8 patients (range 1-53). Overall, the majority of patients (60%) was treatment naive, 52% had advanced fibrosis or cirrhosis and 5% had a history of decompensated liver disease. Baseline characteristics are shown in Table 2. We pooled telaprevir (n=265) and boceprevir (n=202) data, as there were no significant differences in characteristics and treatment outcomes between these patients (S3 and S4 Tables).

Figure 1. Study flowchart



The flowchart shows both enrollment of patients in all centers and assessment of eligibility for registration trials in this study.

Table 2. Baseline characteristics

Characteristic	Overall (n= 467)	Eligible (n= 247)	Ineligible (n=220)	p-value
Age, y – mean (range)	51 (19-77)	50 (22-77)	52 (19-70)	0.07
Male sex – n (%)	319 (68)	170 (69)	149 (68)	0.80
White race – n (%)[a]	321 (89)	173 (91)	148 (88)	0.08
HCV genotype – n (%)				0.23
Genotype 1 indeterminate	86 (18)	49 (20)	37 (17)	
Genotype 1a	226 (48)	122 (49)	104 (47)	
Genotype 1b	155 (33)	76 (31)	79 (36)	
Previous response[b]				0.81
Naive	273 (60)	142 (59)	131 (62)	
Relapse	76 (17)	45 (19)	31 (15)	
Nonresponse	78 (17)	41 (17)	37 (18)	
Viral breakthrough	16 (4)	9 (4)	7 (3)	
Early discontinuation	11 (2)	5 (2)	6 (3)	
Current or history of decompensated liver disease – n (%)	24 (5)	0 (0)	24 (11)	<0.001
Metavir score F3-4[c]	161 (52)	66 (42)	95 (63)	<0.001
Laboratory values[d]				
Hemoglobin g/dL – mean (SD)	9.1 (0.9)	9.2 (0.8)	9.0 (1.0)	0.02
Leucocyte count x10^9/L – mean (SD)	6.7 (2.2)	7.0 (2.1)	6.4 (2.2)	0.003
Neutrophil count x10^9/L – mean (SD)	3.5 (1.5)	3.6 (1.5)	3.3 (1.5)	0.22
Platelet count x10^9/L – mean (range)	192 (24-764)	207 (90-388)	175 (24-764)	<0.001
Albumin g/dL – mean (range)	4.1 (2.4-5.1)	4.3 (3.3-5.1)	4.0 (2.4-5.1)	<0.001
Total bilirubin g/dL – median (IQR)	10.0 (7-14)	9 (7-13)	11 (8-16)	<0.001
Child Pugh (CP) score[e]				0.001
A – n (%)	212 (95)	107 (100)	105 (91)	
B – n (%)	11 (5)	0 (0)	11 (10)	
C – n (%)	0 (0)	0 (0)	0 (0)	

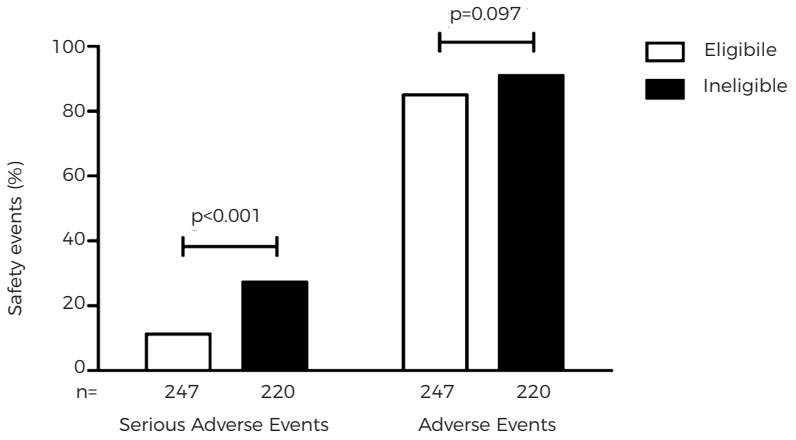
[a] Race: available in 360 patients; [b] Previous response: available in 454 patients; [c] Metavir score: available in 308 patients; [d] Lab values >10% missings in: neutrophil count, albumin; [e] CP-score (assumed no ascites and hepatic encephalopathy at start of treatment): available in 223 patients

Registration trials and outcomes eligible vs. ineligible

Our search yielded eight trials of telaprevir and boceprevir[17-24], and five registration trials were included (S1 Table).[22-24] On the basis of the general criteria (Table 1), 47% of patients treated in real world practice would be excluded from registration trials. We than compared the eligible to ineligible population with respect to safety parameters. We found that ineligible patients had significantly more SAEs compared to eligible patients (27% vs. 11%, p<0.001) (Figure 2). A total of 37 SAEs occurred in 28 eligible patients (1 patient died due to an accident), compared to 103 SAEs which occurred in 60 ineligible patients (7 patients died) (S5 Table). Also, after excluding patients with a history of decompensated liver disease (n=24) from the analysis, ineligible patients had significantly higher SAE rates (24% vs. 11%, p<0.001). Further, ineligible patients had a higher median number of AEs and SAEs (p=0.039 and p<0.001 respectively, S6 Table). The incidence of some typical hepatic or therapy related (S)AEs (anemia, thrombopenia

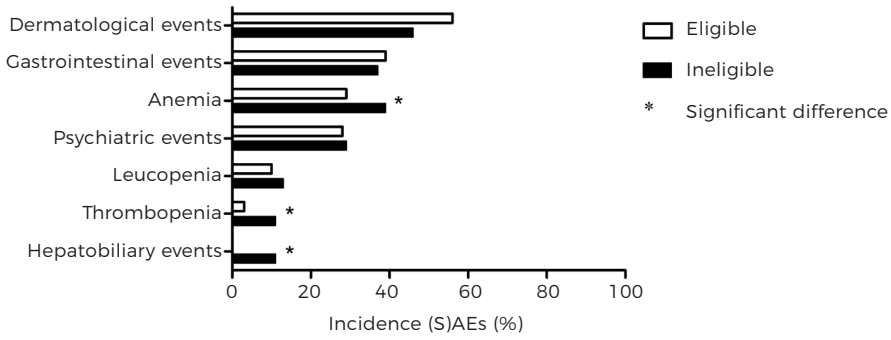
and hepatobiliary events) were significantly higher in the ineligible patients (Figure 3). We found (non-significant) lower SVR rates in ineligible patients. Two sensitivity analyses detected lower SVR rates in ineligible patients (treatment naive – relapse group): when applying most strict criteria (81% vs. 67%, p= 0.01) or when most stringent exclusion of patients with co-morbidity was done (76% vs. 65%, p= 0.02). We observed no difference in SVR in the third sensitivity analysis, where we excluded concomitant medication from the criteria set (Figure 4). No significant differences in effectiveness were found in the non-responder group (S1 Figure).

Figure 2. Safety in real world patients who would be eligible and ineligible for registration trials



The bars represent the proportion of patients who experienced a serious adverse event or adverse event in patients eligible or ineligible for registration trials

Figure 3. Incidence of specific (serious) adverse events in eligible and ineligible patients

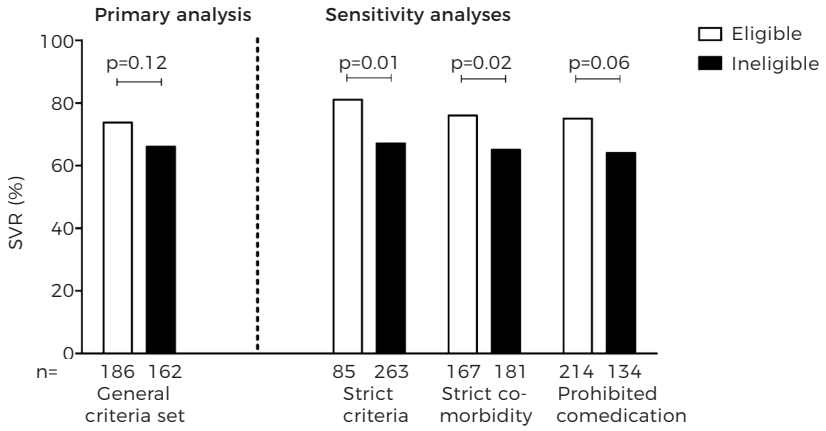


The bars represent the incidence of various categories of (serious) adverse events between patients eligible and ineligible for registration trials. The asterix (*) marks significant differences between eligible and ineligible patients.

Criteria for ineligibility

Most important criteria for ineligibility were related to co-morbidity and signs or history of hepatic decompensation. In 220 ineligible patients, main reason for exclusion was the use of prohibited concomitant medication (n=65), followed by anemia (n=25), psychiatric co-morbidity (n=24), and current or history of decompensated liver disease (n=24). Median number of exclusion criteria within a patient was 1 (range 1-6). Univariable analysis showed most important criteria associated with lack of SVR, i.e. current or history of decompensated liver disease (RR 0.66), platelet count (RR 0.58), albumin (RR 0.49), bilirubin (RR 0.58) and neutrophil count (RR 0.55). Similar criteria were associated with a higher risk on an SAE: a history of decompensated liver disease (RR 1.81), platelet count (RR 1.45), albumin (RR 2.03), bilirubin (RR 1.89), hemoglobin (RR 1.72), malignancy (RR 2.31) and presence of cardiac disease (RR 1.97). Outcomes of these analyses are depicted in Table 3.

Figure 4. Effectiveness in real world treatment naive and relapse patients who would be eligible and ineligible for registration trials



Primary and sensitivity analyses on effectiveness of therapy in eligible vs. ineligible treatment naive and relapse patients (n=348). The bars represent the proportion of patients who reached a sustained virological response (SVR) within the groups. For sensitivity analyses different criteria sets are used to determine eligibility of patients, hence different numbers of patients in both groups.

Discussion

This study sheds doubt on the generalizability of registration trials to the real world CHC population. In our study, one of the key findings is that nearly half of treated CHC patients would be ineligible for registration trials. Most important exclusion criteria relate to signs or history of hepatic decompensation and co-morbidity (cardiac disease, anemia, malignancy and neutropenia). Patients meeting those exclusion

Table 3. Top criteria which impact trial eligibility

Criterion	n	% of ineligible patients	RR on SVR (95% CI)	RR on SAE (95% CI)
Prohibited comedication listed in protocols	65	30	0.99 (0.70-1.39)	1.17 (1.00-1.38)
Hemoglobin <12 g/dL (females) or <13 g/dL (males)	25	11	0.69 (0.46-1.02)	1.72 (1.14-2.60)
Presence of severe psychiatric disease	24	11	1.27 (0.67-2.40)	1.03 (0.84-1.72)
Current or history of ascites, encephalopathy or bleeding varices	24	11	0.66 (0.44-0.97)	1.81 (1.17-2.81)
Platelet count <90 x10^9/L	23	11	0.58 (0.41-0.82)	1.45 (1.01-2.08)
Presence of hemophilia	23	11	1.42 (0.71-2.85)	4.51 (0.66-30.93)*
Serum albumin <3.3 g/dL	22	10	0.49 (0.36-0.68)	2.03 (1.23-3.37)
Total bilirubin >1,8 xULN†	16	7	0.58 (0.39-0.86)	1.89 (1.08-3.29)
TSH >1.2 xULN or <0.8 xLLN†	14	6	0.71 (0.41-1.22)	1.34 (0.36-4.90)*
Active malignant disease or malignant disease in past 5 years (except basal cell carcinoma)	14	6	1.02 (0.50-2.09)	2.31 (1.14-4.66)
Central nervous system disorder present	13	6	0.78 (0.43-1.43)	1.18 (0.82-1.70)
Significant cardiac disease present	12	6	1.46 (0.54-3.91)	1.97 (1.01-3.86)
Presence of auto-immune disease	11	5	1.34 (0.51-3.55)	1.50 (0.87-2.58)
Absolute neutrophil count <1.2 x10^9/L	9	4	0.55 (0.34-0.90)	1.62 (0.25-10.43)*
Ultrasound with no signs of HCC	6	3	0.74 (0.33-1.66)	1.64 (0.74-3.65)
Creatinine clearance ≤50 ml/min	5	2	0.63 (0.30-1.30)	2.05 (0.70-6.01)
AST 10 xULN†	5	2	0.61 (0.29-1.26)	1.01 (0.65-1.57)
Presence of another liver disease	5	2	0.60 (0.29-1.24)	-

* Poisson regression when log binomial regression did not converge; † ULN = upper limit of normal; LLN = lower limit of normal

criteria developed more SAEs (RR between 1.45 and 2.31) and were less likely to reach SVR (RR between 0.49 and 0.66), especially when strict criteria were used. Vice versa, eligible patients had SVR and SAE rates comparable to published trials.[17-21] Altogether, this indicates that results from registration trials are only generalizable to the real world patients who fulfill the eligibility criteria. Translating results originating from registration trials to patients that would be ineligible should be done with caution.

The difference between registration trials and real world reflects a 'development paradox'. Drugs are developed through a phase II-III program that targets easy-to-treat patients, while in the real world difficult-to-treat patients are prioritized for treatment.[1, 25, 26] The sequence of drug development starting with easy-to-treat patients seems appropriate, but the final hurdle to perform trials that specifically target difficult-to-treat patients is often sidestepped or delayed until after market authorization.

As a result, this population who has a clear treatment indication is exposed to DAAs in the real world, without proper data on efficacy and toxicity.[27] This results in an increased proportion of adverse events, dropouts and hence lower effectiveness.[28] Our results support the 'development paradox' and provide reasons why real world outcomes do differ from registration trials.

Our data on limited generalizability of registration trials accords with the literature. An increased likelihood for SAEs in patients with a history of decompensated cirrhosis who would have been excluded from registration trials was reported in a large CHC cohort (n=2084).[9] Some 30-47% of compensated cirrhotic patients treated with first-generation protease inhibitors would be ineligible for registration trials, and this study showed unexpected high SAE rates in that population.[7]
In addition, a study on ledipasvir/sofosbuvir in advanced liver disease patients, published after FDA and EMA approval, reported much higher SAE rates (23%) compared to registration trials (3%).[29] For another CHC regimen, paritaprevir/ritonavir, ombitasvir and dasabuvir, the FDA label changed within one year following approval based on review of adverse events. This regimen is now contra-indicated in patients with Child-Pugh B cirrhosis.[30] It is likely that this could have been prevented if these patients had been trialed prior to approval of the regimen. There is literature that suggests that serious adverse events might be related to disease course instead of therapy.[31] Nonetheless, timely controlled studies in CHC patients with decompensated liver disease are necessary to accurately gauge risk-benefit balance for these individual patients.

Here, we used the first-generation protease inhibitor treated patients as an example cohort. We believe that our results are also applicable to new generation DAAs, because eligibility criteria of registration trials are comparable to the set used in the current study (S7 Table).[31-37] Indeed, a Canadian HIV/HCV cohort, found that up to 94% of patients from that cohort would be ineligible for registration trials with new generation DAAs.[10] Furthermore a real world cohort showed that liver decompensation and SAEs during sofosbuvir containing regimens were associated with lower baseline albumin and higher total bilirubin, which are general exclusion criteria.[38] As toxicity of new generation DAAs decreases, the difference between trials and real world might become smaller, however with the high ineligibility rate of real world patients, generalization of results remains difficult.

Limited generalizability of registration trials is also seen in other liver diseases such as hepatocellular carcinoma (HCC) and HBV infection. For example, sorafenib was approved for HCC treatment, on the basis of studies that excluded Child-Pugh B and C cirrhotic patients.[39, 40] A real world cohort reported significantly decreased overall survival with sorafenib in Child-Pugh B compared to Child-Pugh A cirrhotics.[41] Likewise, post-marketing studies in entecavir for chronic HBV infection show lower proportions of ALT normalization than was shown in registration trials.[42]

Our study comes with strengths and limitations. Strengths of this study are the nationwide and multicenter character, resulting in a large and representative real world cohort. Limitations of this study are the retrospective character that resulted in (some) missing values. We handled this conservatively, by classifying the missing value as eligible for that criterion. Furthermore, chart review may result in reporting bias, but we used strict definitions to reduce this. Another limitation is that patients received

first-generation protease inhibitors, peginterferon and ribavirin, which may increase the potential for toxicity. However, we think that our results are also valid for new generation DAAs.

In conclusion, nearly half of CHC patients treated in real world practice would be ineligible for registration trials. In these patients we found impaired safety and effectiveness related to specific eligibility criteria (hepatic decompensation and co-morbidity). Prior to regulatory approval, new drugs should also be studied in the difficult-to-treat population, including patients with hepatic decompensation and co-morbidity, to genuinely assess the benefits and risks of treatment in the real world population.

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Collaborators

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Abbreviations

AE	Adverse Event
CHC	Chronic Hepatitis C
CNS	Central Nervous System
CTCAE	Common Terminology Criteria for Adverse Events
DAA	Direct-Acting Antiviral
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
INR	International Normalized Ratio
LLN	Lower Limit of Normal
RNA	RiboNucleic Acid
RR	Relative Risk
SAE	Serious Adverse Event
SVR	Sustained Virological Response
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal

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Supporting Information

S1 Table. Search strategy

This is the flowchart of the systematic search for registration trials with telaprevir and boceprevir.

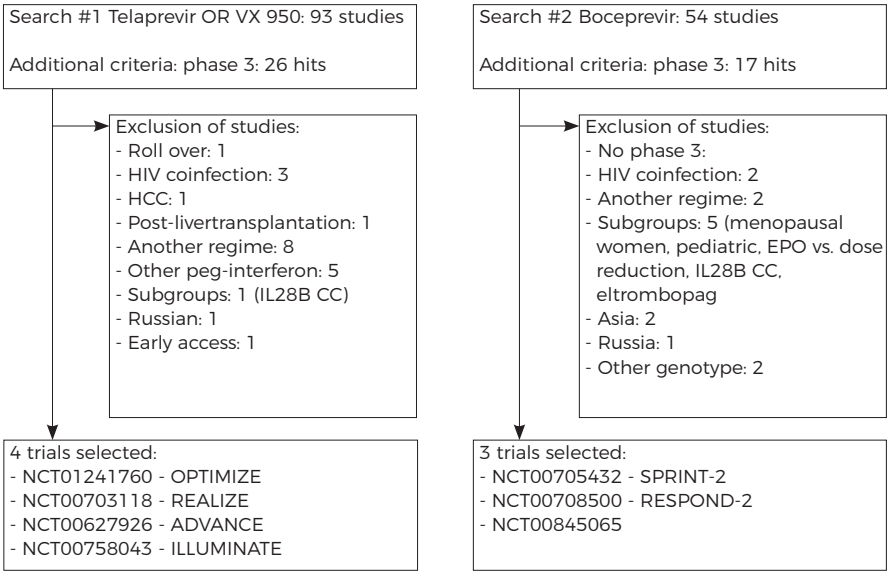
FDA Telaprevir label (Reference ID: 3397093), clinical studies:
- Treatment naive adults: trial 108 (ADVANCE), trial 111 (ILLUMINATE), trial C211 (OPTIMIZE)
- Treatment experienced adults: trial C216 (REALIZE)

FDA Boceprevir label (Revised 05/2011), clinical studies:
- Treatment naive adults: SPRINT-2
- Treatment experienced adults: RESPOND-2

Pubmed database

No	Search	Results	Exclusion
#1	Telaprevir AND phase 3 Limits: - clinical trial - date: 1-1-2000 until 1-1-2014	31 results: Inclusion: - NCT01241760 – OPTIMIZE - NCT00703118 – REALIZE - NCT00627926 - ADVANCE	Exclusion on title and abstract: 27 Exclusion on full text: 1 (Japan)
#2	Boceprevir AND phase 3 Limits: - clinical trial - date: 1-1-2000 until 1-1-2014	25 results Inclusion: - NCT00910624 - PROVIDE - NCT00845065 - NCT00705432 - SPRINT-2 - NCT00708500 - RESPOND-2	Exclusion on title and abstract: 21

Clinical trials.gov



Conclusion search in FDA label, clinical trials.gov and pubmed:

	Telaprevir	Boceprevir
Inclusion	- NCT00703118 – REALIZE - NCT00627926 – ADVANCE - NCT00758043 - ILLUMINATE	- NCT00705432 - SPRINT-2 - NCT00708500 - RESPOND-2
Exclusion	- NCT01241760 – OPTIMIZE: no full eligibility criteria available	- NCT00910624 – PROVIDE: roll over of SPRINT-1, SPRINT-2 and RESPOND-2, so identical inclusion criteria - NCT00845065 (identical inclusion criteria RESPOND-2)

S2 Table. Set of least and most stringent combined inclusion and exclusion criteria of registration trials

This table shows the least stringent and most stringent criteria of different registration trials per variable. The least stringent criteria set was used for primary analyses and the most stringent criteria set for a sensitivity analysis.

Variable	Least stringent criteria	Most stringent criteria
Inclusion		
Age	Subject ≥ 18 years	Subject 18-70 years
HCV RNA	HCV RNA detectable	HCV RNA ≥ 10.000
Weight	Between 40-125 kg	Between 40-125 kg
Hepatocellular carcinoma (HCC)	Ultrasound with no signs of HCC	Ultrasound with no signs of HCC
Exclusion		
Genotype	HCV with > 1 subtype or genotype	HCV with > 1 subtype or genotype
Treatment history	Ignore this criterion	Exclusion of nullresponders, viral breakthrough and early discontinuation
Hemoglobin (Hb)	Hb <12 g/dL (females) or <13 g/dL (males)	Hb <12 g/dL (females) or <13 g/dL (males)
Neutrophil count	Absolute neutrophil count <1.2x10^9/L	Absolute neutrophil count <1.5x10^9/L
Platelet count	Platelet count <90 x10^9/L	Platelet count <100 x10^9/L
Renal insufficiency	Creatinine clearance ≤ 50 ml/min	Creatinine > ULN†
Albumin	Serum albumin < 3.3 g/dL	Serum albumin < LLN†
Bilirubin	total bilirubin > 1.8 xULN†	total bilirubin > 1.6 xULN †
Glucose	Ignore this criterion	Serum glucose ≥ 140 mg/dL (nonDM)
Protrombin Time (PT)/ INR	INR ≥ 1.5	PT > 10% ULN†
TSH	TSH > 1.2 xULN or < 0.8 xLLN†	TSH above or below normal range
ALT	ALT 10 xULN†	ALT 10 xULN†
AST	AST 10 xULN†	AST 10 xULN†
Contra-indication to peginterferon/ribavirin		
- Hemoglobinopathy	Hemoglobinopathy present	Hemoglobinopathy present*
- Cardiac disease[1]	Significant cardiac disease present	Significant cardiac disease present*
- Renal insufficiency	See renal insufficiency	See renal insufficiency*
Auto-immune disease[2]	Presence of auto-immune disease	Presence of auto-immune disease*
COPD	COPD gold III or IV	COPD gold I-IV and unknown gold

Variable (continued)	Least stringent criteria (continued)	Most stringent criteria (continued)
Exclusion (continued)		
Current or history of decompensated liver disease	History of ascites, encephalopathy or bleeding varices	History of ascites, encephalopathy or bleeding varices*
Other liver disease	Other liver disease	Other liver disease*
Malignancy	Active malignant or malignant disease in past 5 years (except basal cell carcinoma)	Active malignant or malignant disease in past 5 years (except basal cell carcinoma)*
Pancreatitis	Acute pancreatitis in past 5 years	History of acute or chronic pancreatitis
Retinopathy	Retinopathy present	Retinopathy present*
Seizure	Seizure disorder requiring medication	History of seizure disorder
Transplantation	Patient with a history of an organ transplant	Patient with a history of an organ transplant*
Psychiatric comorbidity[3]	Severe psychiatric disease	Moderate and severe psychiatric disease
Corticosteroids use	Use of systemic corticosteroids	Use of systemic corticosteroids*
Alcohol or drugs use	Ignore this criterion	Alcohol use > 2 IE/day or drugs use
Hemophilia	Hemophilia	Hemophilia*
Central Nervous System disorder/Stroke/TIA[4]	CNS disorder present	CNS disorder present*
Malabsorption	History of malabsorption disorder	History of malabsorption disorder*
Indwelling cathether	Subject with indwelling venous catheter	Subject with indwelling venous catheter*
Comedication	Comedication literally on prohibited medication list of protocol	Comedication in the same anatomical therapeutic code (ATC)-group as prohibited medication list of protocol

[1] Significant cardiac disease was defined as: current or history of unstable cardiac disease (angina, congestive heart failure, recent myocardial infarction, pulmonary hypertension, complex congenital heart disease, cardiomyopathy, and/or significant arrhythmia)

[2] Auto-immune disease was defined as: immunologically mediated disease (inflammatory bowel disease, celiac disease, rheumatoid arthritis, idiopathic thrombocytopenic purpura, systemic lupus erythematosus, auto-immune hemolytic anemia, scleroderma, sarcoidosis, severe psoriasis, or auto-immune hepatitis)

[3] Psychiatric comorbidity was defined as: severe depression or hospitalization for depression, schizophrenia, bipolar illness, severe anxiety or personality disorder, a period of disability or impairment due to a psychiatric disease within the past 5 years

[4] CNS disorder was defined as: CNS trauma requiring intubation, intracranial pressure monitoring, brain meningeal/skull surgery, or resulting in seizure, coma, neurologic deficits, abnormal brain imaging, CSF leak, prior brain hemorrhage and/or intracranial aneurysms, or history of stroke or transient ischemic attack (TIA)

† ULN = upper limit of normal; LLN = lower limit of normal

* plus exclusion of cases with insufficient information about specific co-morbidity

S3 Table. Baseline characteristics telaprevir and boceprevir treated patients

Table including baseline characteristics of patients treated with telaprevir and boceprevir, these patients are pooled in the primary analysis.

Characteristic	Telaprevir (n=265)	Boceprevir (n=202)	p-value (TPR/BOC)
Age, y – mean (range)	51 (19-71)	51 (22-77)	0.84
Male sex – n (%)	174 (66)	145 (72)	0.16
White race – n (%)[a]	186 (89)	135 (90)	0.43
HCV genotype – n (%)			0.35
Genotype 1 indeterminate	31 (20)	33 (16)	
Genotype 1a	121 (46)	105 (52)	
Genotype 1b	91 (34)	64 (32)	
Previous response[b]			0.70
Naive	152 (59)	121 (61)	
Relapse	40 (15)	36 (18)	
Nonresponse	47 (18)	31 (16)	
Viral breakthrough	11 (4)	5 (3)	
Early discontinuation	7 (3)	4 (2)	
Decompensated liver disease – n (%)	12 (5)	12 (6)	0.49
Metavir score F3-4[c]	96 (52)	65 (52)	0.97
Laboratory values[d]			
Haemoglobin g/dl – mean (SD)	9.1 (0.9)	9.2 (0.9)	0.29
Leucocyte count $x10^9$/L – mean (SD)	6.8 (2.1)	6.7 (2.2)	0.74
Neutrophil count $x10^9$/L – mean (SD)	3.5 (1.6)	3.4 (1.5)	0.66
Platelet count $x10^9$/L – mean (range)	195 (51 – 764)	188 (24 – 387)	0.31
Albumin g/dL – mean (range)	4.2 (2.4-5.1)	4.1 (2.4-4.9)	0.06
Total bilirubin g/dl – median (IQR)	10 (7-14)	9.9 (7-14)	0.82

[a] Race: available in 360 patients; [b] Previous response: available in 454 patients (257 TPR, 197 BOC); [c] Metavir score: available in 308 patients (184 TPR, 124 BOC); [d] Lab values >10% missings in: neutrophil count, albumin.

S4 Table. Effectiveness and safety of telaprevir compared to boceprevir

Table showing effectiveness and safety results of telaprevir vs. boceprevir, these patients are pooled in the primary analysis.

	TPR (n=265)	BOC (n=202)	p-value
SVR on previous response, n (%)			
Naive (TPR n=152, BOC n=121)	107 (70)	84 (69)	0.86
Relapse (TPR n=40, BOC n=36)	27 (69)	26 (72)	0.78
Non response (TPR n=47, BOC n=31)	20 (43)	13 (42)	0.96
Viral breakthrough (TPR n=11, BOC n=5)	4 (36)	1 (20)	1.00*
Early discontinuation (TPR n=7, BOC n=4)	5 (71)	1 (25)	0.24*
Unknown (TPR n=8, BOC n=5)	5 (63)	3 (60)	1.00*
SVR on previous response, n (%)			
Naive and Relapse (n=349)	135 (70)	110 (70)	0.96
Nonresponse, viral breakthrough, early discontinuation (n=118)	34 (47)	18 (40)	0.49
SAE on previous response, n (%)			
Naive (TPR n=152, BOC n=121)	33 (22)	18 (15)	0.15
Relapse (TPR n=40, BOC n=36)	9 (23)	4 (11)	0.19
Non response (TPR n=47, BOC n=31)	9 (19)	5 (16)	0.73
Viral breakthrough (TPR n=11, BOC n=5)	1 (9)	3 (60)	0.06*
Early discontinuation (TPR n=7, BOC n=4)	1 (14)	1 (25)	1.00*
Unknown (TPR n=8, BOC n=5)	3 (38)	1 (20)	1.00*
Mean sum AE on previous response (±SD)			
Naive (TPR n=152, BOC n=121)	3.0±2.2	2.8±2.3	0.63
Relapse (TPR n=40, BOC n=36)	2.7±1.8	3.4±2.2	0.10
Non response (TPR n=47, BOC n=31)	3.0±2.5	2.7±2.9	0.82
Viral breakthrough (TPR n=11, BOC n=5)	1.9±2.1	3.6±3.2	0.36
Early discontinuation (TPR n=7, BOC n=4)	2.7±0.8	2.8±1.0	0.58
Unknown (TPR n=8, BOC n=5)	3.0±1.5	3.2±2.1	0.83
Mean sum SAE on previous response (±SD)			
Naive (TPR n=152, BOC n=121)	0.3±0.7	0.25±0.7	0.09
Relapse (TPR n=40, BOC n=36)	0.3±0.5	0.17±0.5	0.27
Non response (TPR n=47, BOC n=31)	0.2±.05	0.48±1.5	0.02
Viral breakthrough (TPR n=11, BOC n=5)	0.3±0.9	1.00±1.0	0.41
Early discontinuation (TPR n=7, BOC n=4)	0.1±0.4	0.25±0.5	0.45
Unknown (TPR n=8, BOC n=5)	0.6±0.9	0.40±0.9	0.59

* analysis with Fisher exact as frequency counts <5

S5 Table. Serious adverse events categories

Table with categories of serious adverse events in eligible vs. ineligible patients

Category[1]	Eligible[2] (37 SAEs)	Number of eligible patients (n=28)	Ineligible[3] (103 SAEs)	Number of ineligible patients (n=60)	p-value (Fisher exact)
Hepatobiliary	1 (2.7)	1 (3.6)	23 (22.3)	15 (25.0)	0.017
Anemia	4 (10.8)	3 (10.7)	15 (14.6)	14 (23.3)	0.247
Respiratory	9 (24.3)	8 (28.6)	13 (12.6)	10 (16.7)	0.257
Gastrointestinal	5 (13.5)	4 (14.3)	9 (8.7)	7 (11.7)	0.738
Circulatory	0 (0.0)	0 (0.0)	8 (7.8)	8 (13.3)	0.051
Psychiatric	3 (8.1)	3 (10.7)	6 (5.8)	5 (8.3)	0.706
General	3 (8.1)	3 (10.7)	6 (5.8)	6 (10.0)	1.00
Central nervous system	0 (0.0)	0 (0.0)	5 (4.9)	4 (6.7)	0.302
Endocrine	1 (2.7)	1 (3.6)	4 (3.9)	3 (5.0)	1.00
Musculoskeletal	1 (2.7)	1 (3.6)	4 (3.9)	3 (5.0)	1.00
Skin	4 (10.8)	3 (10.7)	3 (2.9)	3 (5.0)	0.378
Ear Nose Throat	1 (2.7)	1 (3.6)	3 (2.9)	2 (3.3)	1.00
Renal	2 (5.4)	2 (7.1)	2 (1.9)	2 (3.3)	0.589
Leucopenia	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.7)	1.00
Pancytopenia	1 (2.7)	1 (3.6)	0 (0.0)	0 (0.0)	0.318
Nutritional	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.7)	1.00
Reproductive system	1 (2.7)	1 (3.6)	0 (0.0)	0 (0.0)	0.318
Eye disorder	1 (2.7)	1 (3.6)	0 (0.0)	0 (0.0)	0.318

[1] Percentage is noted between brackets in whole table

[2] One eligible patient died due to an accident

[3] Seven ineligible patients died, causes: hepatic encephalopathy, decompensated liver disease and CVA, sepsis and CVA, CVA, renal insufficiency, endocarditis, and one patient died of unknown cause

S6 Table. Sensitivity analyses

Table showing outcomes of sensitivity analyses: analysis with most stringent criteria, analysis with strict exclusion of patients with co-morbidity, analysis without prohibited comedication as exclusion criterion

Sensitivity analysis		Eligible – n (%)	Ineligible – n (%)	p-value
Primary analysis	No of patients – n (%)	247 (53)	220 (47)	
	SVR (TN/relapse, n=348) – n (%)	137/186 (74)	107/162 (66)	0.12
	SVR (NR/other, n=118) – n (%)	29/60 (48)	23/58 (40)	0.34
	SAE – n (%)	28 (11)	60 (27)	<0.001
	Sum of SAEs – median (IQR)	0 (0-0)	0 (0-1)	0.001*
	AE – n (%)	211 (85)	199 (91)	0.097
	Sum of AEs – median (IQR)	2 (1-4)	3 (1-5)	0.039*
Sensitivity analysis: most strict analysis	No of patients – n (%)	102 (22)	365 (78)	
	SVR (TN/relapse, n=348) – n (%)	69/85 (81)	175/263 (67)	0.01
	SVR (NR/other, n=118) – n (%)	7/16 (44)	45/102 (44)	0.98
	SAE – n (%)	12 (12)	76 (21)	0.04
	Sum of SAEs – median (IQR)	0 (0-0)	0 (0-0)	0.034*
	AE – n (%)	84 (82)	326 (89)	0.06
	Sum of AEs – median (IQR)	2 (1-4)	3 (1-4)	0.018*
Sensitivity analysis: more strict exclusion of patients with co-morbidity	No of patients – n (%)	222 (48)	245 (52)	
	SVR (TN/relapse, n=348) – n (%)	127/167 (76)	117/181 (65)	0.02
	SVR (NR/other, n=118) – n (%)	26/54 (48)	26/64 (41)	0.41
	SAE – n (%)	23 (10)	65 (27)	<0.001
	Sum of SAEs – median (IQR)	0 (0-0)	0 (0-1)	<0.001*
	AE – n (%)	188 (85)	222 (91)	0.05
	Sum of AEs – median (IQR)	2 (1-4)	3 (1-5)	0.001*
Sensitivity analysis: comedication not included in analysis	No of patients – n (%)	289 (62)	178 (38)	
	SVR (TN/relapse, n=348) – n (%)	158/214 (75)	86/134 (64)	0.06
	SVR (NR/other, n=118) – n (%)	34/74 (46)	18/44 (41)	0.59
	SAE – n (%)	38 (13)	50 (28)	<0.001
	Sum of SAEs – median (IQR)	0 (0-0)	0 (0-1)	<0.001*
	AE – n (%)	253 (88)	157 (88)	0.83
	Sum of AEs – median (IQR)	2 (1-4)	3 (1-5)	0.030*

* analysis performed with Mann-Whitney U test

TN= Treatment Naive; NR= Non responder; SAE= Serious Adverse Event; SVR= Sustained Virological Response; AE = Adverse Event

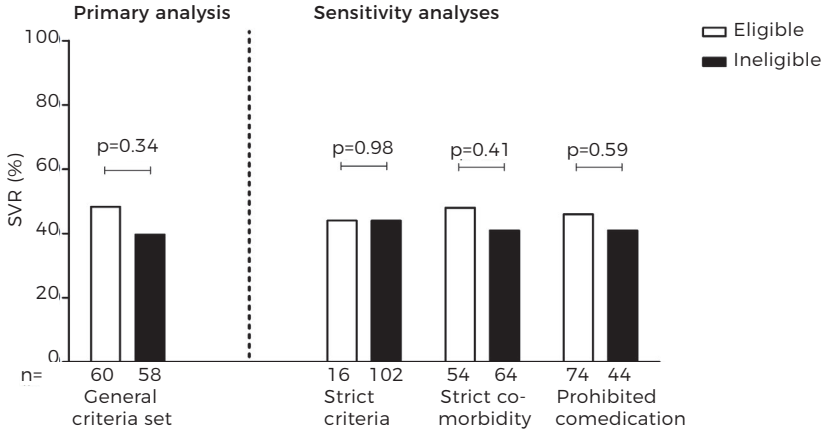
S7 Table. Exclusion criteria of registration trials in new generation DAAs

Table with exclusion criteria of new generation DAAs in comparison to our general criteria set

General exclusion criteria set of our study	Ledipasvir/sofosbuvir		Paritaprevir/ritonavir, ombitasvir, dasabuvir	
	ION-1, ION-II	ION-III	Sapphire I, Pearl III, Pearl IV, Saphire II, Pearl II	Turquoise II (compensated cirrhotics)
Decompensated liver disease	Clinical hepatic decompensation	Presence of cirrhosis	Presence of cirrhosis	Child Pugh-B, and -C cirrhosis
Platelets < 90 x10^9/L	Platelets < 50 x10^9/L	Platelets < 90 x10^9/L	Platelets < 120 x10^9/L	Platelets < 60 x10^9/L
Total bili > 1.8 xULN†	Direct bili > 1.5 xULN†	Direct bili > ULN†	Indirect bili >1.5 ULN and direct bili > ULN†	Total bili ≥ 3.0 mg/dL
Serum albumin < 3.3 g/dL	Serum albumin < 3.0 g/dL	Serum albumin < 3.0 g/dL	Serum albumin < LLN†	Serum albumin < 2.8 g/dL
Significant cardiac disease	Significant cardiac disease	Significant cardiac disease	Significant cardiac disease	Significant cardiac disease
Hb <12 g/dL (females) or <13 g/dL (males)	Hb <11 g/dL (females) and <12 g/dL (males)	Hb <11 g/dL (females) and <12 g/dL (males)	Hb < LLN†	Hb < LLN†
Active or recent malignancy	Active or recent malignancy	Active or recent malignancy	Active or recent malignancy	Active or recent malignancy
Absolute neutrophil count <1.2 x10^9/L	n/a	n/a	Absolute neutrophil count <1.5 x10^9/L	Absolute neutrophil count <1.5 x10^9/L

† ULN = upper limit of normal; LLN = lower limit of normal; DAA = Direct-Acting Antiviral

S1 Figure. Effectiveness in real world treatment nonresponder and other patients who would be eligible and ineligible for registration trials



Primary and sensitivity analyses on effectiveness of therapy in eligible vs. ineligible nonresponder or other patients (n=118). For sensitivity analyses different criteria sets are used to determine eligibility of patients, hence different numbers of patients in groups.

High risk of infection during triple therapy with first-generation protease inhibitors: a nationwide cohort study

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Abstract

Background and Aims

Peginterferon (pegIFN) remains the backbone of therapy for chronic hepatitis C (CHC) in economically constrained regions. However, pegIFN may cause neutropenia and addition of a protease inhibitor can increase the likelihood of neutropenia. The aims of this study were to assess the occurrence of clinically relevant infections during first-generation protease inhibitor based therapy and its risk factors as well as the relation to treatment-induced neutropenia.

Methods

This multicenter (n=45) retrospective cohort study included CHC patients treated in the Netherlands. Based on absolute neutrophil count, categories of neutropenia were defined: severe (<500/μL), moderate (500-750/μL) and mild (750-1500/μL). Likewise, infections were classified as severe (intravenous antibiotics/hospitalization) and moderate (anti-infective treatment). We assessed risk factors for infections using multivariable regression analysis with correction for multiple measurements.

Results

We included 467 CHC patients, 319 (68%) were male and 111 (24%) had cirrhosis. A total of 185 clinically relevant infections (34 severe) occurred in 145 patients (31%). During treatment 310 patients experienced neutropenia (34 severe). Multivariable analysis identified female sex (OR 1.7, 95%CI 1.2-2.5), COPD (OR 2.7, 95%CI 1.6- 4.5) and diabetes mellitus (OR 1.7, 95%CI 1.0-3.0) as risk factors for infections. Neutropenia at the previous visit was not associated with infection (univariable analysis: OR 0.9, 95%CI 0.6-1.3).

Conclusion

This study shows that therapy with first-generation protease inhibitors was complicated by an infection in 31% of patients. Not neutropenia, but female sex, COPD and diabetes mellitus were independent risk factors for infection. These patients should be monitored carefully once a pegIFN regimen is initiated.

Introduction

For many years pegylated interferon-α (pegIFN) has been the backbone of chronic hepatitis C (CHC) treatment. The introduction of first-generation direct acting antivirals (DAAs), telaprevir and boceprevir, initiated a cascade of developments of new generation DAAs.[1] From 2014 onwards, pegIFN-free treatment options with higher cure rates and better tolerability have become available in many western countries.[2] These new pegIFN-free regimens are very costly, limiting the availability in many economically deprived regions worldwide, where the majority of the global CHC population resides.[3-6] Guidelines still recommend telaprevir and boceprevir for use in countries where new generation DAAs are not available. Therefore, triple therapy still maintains its therapeutic value.[7, 8]

One of the drawbacks of triple therapy is its high rate of adverse events, which often can be attributed to the use of pegIFN. Neutropenia is frequently reported and mainly caused by bone marrow suppression.[9, 10] To prevent infections, product labels and guidelines advise dose reductions or even discontinuation of treatment if neutrophil count drops below 750/μL or 500/μL respectively.[7, 11] However, prior studies in CHC patients undergoing (peg)IFN and ribavirin (RBV) therapy did not find an association between treatment-induced neutropenia and infections, while dose reductions of pegIFN can reduce effectiveness.[12-17] The situation may be different with triple therapy, because phase III studies found that the inclusion of boceprevir to the CHC treatment strategy increases the likelihood of neutropenia compared to pegIFN and RBV.[18, 19] In addition, comparative studies found more neutropenia in boceprevir than telaprevir treated patients.[20, 21] Real world data furthermore suggest that triple therapy substantially increases the risk of severe infections. However, the current evidence for this association is limited to CHC patients with cirrhosis.[22-24] Therefore, the aims of this study were (i) to investigate the occurrence and risk factors for clinically relevant infections and (ii) the relation of on-treatment neutropenia with infections in CHC patients who received triple therapy with boceprevir or telaprevir.

Methods

Population and design

This nationwide, multicenter, real world cohort study included patients with a CHC genotype 1 infection treated with telaprevir or boceprevir and pegIFN and RBV in the Netherlands (2011-2015) [unpublished data]. Patients across all fibrosis stages were included. We retrospectively identified patients from local databases, and excluded patients with a co-infection with human immunodeficiency virus or hepatitis B virus. Treatment choice between telaprevir and boceprevir was at the discretion of the physician and it was administered according to national guidelines.[25] We conducted the study in accordance with good clinical practice (GCP) guidelines, and the code of

conduct for medical research (www.federa.org). Approval from participating centers was obtained following local regulations.

Outcomes and definitions

The primary outcome of this study was occurrence of infections during treatment until 4 weeks after cessation of treatment. Secondary outcomes were occurrence and severity of neutropenia, risk factors for infection, and severity of infection. In addition the time until occurrence of the first infection after start of treatment was assessed. Infections were classified as severe in case of hospitalization or intravenous antibiotics, moderate if oral or topical anti-infective agents were administered and mild if no treatment was given. Moderate and severe infections were considered clinically relevant. Based on the thresholds for dose reduction and treatment discontinuation of pegIFN, we distinguished three categories of neutropenia: severe if absolute neutrophil count (ANC) was below 500/μL, moderate if ANC was between 500 and 750/μL and mild if ANC was between 750 and 1500/μL.[13, 25] We used Fib-4 > 3.25 to classify patients as cirrhosis, because of high performance in detecting cirrhosis and high availability of included biomarkers in the general population.[26] History of decompensated liver disease was defined as a history of ascites, variceal bleeding or hepatic encephalopathy.

Data acquisition

We collected all details on demographics, disease characteristics, infectious (serious) adverse events, and laboratory values. Laboratory values included hematological tests, creatinine, aminotransferases, and indicators of liver function. In case two infections occurred within the same timeframe, we only included the most severe infection.

Statistical analysis

We described categorical variables as proportions and continuous variables as means (standard deviation, SD) or medians (interquartile range, IQR). The Kaplan-Meier method was used to assess time till occurrence of the first infection and the cumulative incidence rates of infections at 12 and 24 weeks after treatment initiation. Those time points were chosen as the introduction of new generation DAAs allows shortened use of pegIFN for 12 or 24 weeks.[7] Chi-square tests were performed to compare occurrence of at least one clinically relevant infection between subgroups of patients with and without diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD) and cirrhosis. To identify predictors for clinically relevant infections we performed univariable and multivariable logistic regression analyses with correction for multiple measurements within a patient. Variables with a p-value ≤0.2 in univariable analysis were included in multivariable analysis together with age, sex, cirrhosis and DM as fixed factors (backward stepwise method, complete cases). ANC at the visit prior to the occurrence of infection was included in univariable analysis. Odds ratios (OR) with 95% confidence intervals (95%CI) are reported. As a sensitivity analysis, all reported infections were included.

All analyses included the intention to treat population, and all tests were two-sided with a significance level of $p < 0.05$. The analyses were performed using SPSS (IBM SPSS Statistics 20) and SAS 9.3 (SAS Institute, Cary, NC, USA).

Results

Population

Our cohort study included 489 patients in total, of which 22 were excluded. Therefore 467 patients from 45 centers in the Netherlands were analyzed (Supplementary Figure 1). Patients were treated with telaprevir (n= 265) or boceprevir (n= 202) and pegIFN and RBV. Mean age was 51 years (range 19-77), 319 (68%) patients were male, and 111 (24%) patients presented with cirrhosis. Baseline characteristics are shown in Table 1.

Table 1. Baseline characteristics

Characteristic	Overall (n= 467)	No infection (n =322)	Infection (n =145)
Age in years – mean (range)	51 (19-77)	50 (19-77)	52 (25-74)
Male sex – n (%)	319 (68)	234 (73)	85 (59)
White race – n (%)[a]	321 (89)	225 (89)	96 (91)
HCV genotype – n (%)			
Genotype 1 indeterminate	86 (18)	64 (20)	22 (15)
Genotype 1a	226 (48)	158 (49)	68 (47)
Genotype 1b	155 (33)	100 (31)	55 (37)
Treatment naive – n (%)[b]	273 (60)	190 (61)	83 (58)
Fib 4 index – median (IQR)[c]	1.8 (1.1-3.3)	1.6 (1.1-2.9)	2.1 (1.3-4.1)
Fib 4 > 3.25 – n (%)[c]	111 (25)	67 (22)	44 (32)
History of decompensated liver disease – n (%)	24 (5)	9 (3)	15 (10)
Diabetes Mellitus – n (%)	54 (12)	30 (9)	24 (17)
Chronic Obstructive Pulmonary Disease – n (%)	37 (8)	16 (5)	21 (14)
Telaprevir vs. boceprevir – n	265 vs. 202	186 vs. 136	79 vs. 66
Laboratory values[d]			
Hemoglobin g/dL – mean (SD)	9.1 (0.9)	9.2 (0.9)	9.0 (0.9)
Leucocyte count per µL – mean (SD)	6727 (2154)	6827 (2152)	6500 (2151)
Neutrophil count per µL – mean (SD)	3454 (1532)	3467 (1503)	3423 (1606)
Platelet count $x10^9$/L – mean (SD)	192 (76)	198 (78)	179 (71)
Albumin g/L – mean (SD)	41.4 (4.9)	41.8 (4.6)	40.7 (5.5)
Total bilirubin g/dL – median (IQR)	10.0 (7-14)	10 (7-14)	10 (7-16)

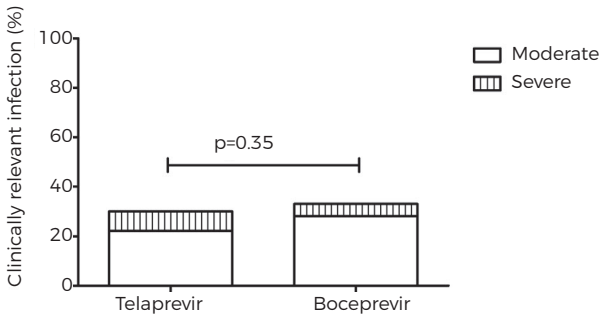
[a] Race: available in 360 patients (252 without infection, 108 with infection)
[b] Previous response: available in 454 patients (310 without infection, 144 with infection)
[c] Fib-4 index: available in 438 patients (301 without infection, 137 with infection)
[d] Lab values >10% missings at baseline: neutrophil count, albumin

Infections

In total, 233 infections in 171 patients were reported (34 severe, 151 moderate, and 47 mild), and thus 185 were clinically relevant occurring in 145 patients (31%). A total of 79 of 265 telaprevir treated patients experienced 103 infections and 66 of 202 boceprevir treated patients experienced 82 infections. Incidence and severity of infections were similar for telaprevir vs. boceprevir (p=0.35, Figure 1). Main sites of infection were dermatological, respiratory, and gastro-intestinal (Table 2). In total, 34 severe infections were observed in 31 patients (21 telaprevir and 10 boceprevir treated patients). Sites and diagnoses of severe infections are listed in Table 3. Among patients with DM, COPD or cirrhosis, more infections were reported than patients without DM, COPD or cirrhosis (DM: 46% vs. 29%, p=0.010; COPD: 57% vs. 29%, p<0.001; cirrhosis 40% vs. 28%, p=0.024; Figure 2)

Infection resulted in death in 2 patients: one patient was admitted with anemia and sepsis (bloodculture: Klebsiella and Staphylococcus Aureus) and died in-hospital while the other patient died from a mycotic endocarditis (bloodculture: Candida Parapsilosis). The median time to develop a clinically relevant infection was 14 weeks (IQR 6-26 weeks). Cumulative incidence of infection within the first 12 weeks was 17.4% (95%CI 12.9-21.9) for telaprevir treatment and 12.6% (95%CI 7.9-17.3) for boceprevir treatment (Figure 3). Overall, no significant differences were seen in the cumulative incidence of infections between telaprevir and boceprevir (p=0.712).

Figure 1. Severity of infection in telaprevir and boceprevir treated patients



The bars represent the percentage of patients who experienced a clinically relevant infection among the patients treated with either telaprevir or boceprevir. A total of 79 patients treated with telaprevir and 66 patients treated with boceprevir experienced an infection.

Neutrophil counts and infections

At baseline mean ANC was 3454/μL (SD 1532) and 21 (of 284 available measurements) patients had none-severe neutropenia. Only 5 (24%) of these patients developed an infection (1 severe). Neutrophil count dropped by an average of 2201/μL (SD 1339)

during treatment. A total of 310 (74%) of 419 patients with available ANC measurements (48 patients had no ANC tests available) experienced neutropenia during treatment. There were more neutropenia episodes in patients treated with boceprevir than with telaprevir (83% vs. 67%, p<0.001), and we detected a trend towards a higher cumulative incidence of severe neutropenia among patients treated with boceprevir (p=0.052, Figure 4). The median time to nadir neutrophil count per patient was 16 weeks (IQR 8-24 weeks), this was similar for both DAAs.

In 127 of 185 (69%) clinically relevant infections, neutrophil count from the previous visit had been recorded and median neutrophil count prior to infection was 1600/μL (IQR 1.1-2.3). Overall, 57 times a clinically relevant infection was diagnosed (moderate n=50; severe n=7) in patients who had neutropenia in the preceding visit (89% mild). By contrast, 1456 visits with neutropenia (96%) were not followed by an infection.

Table 2. Categories of clinically relevant infections (moderate and severe)

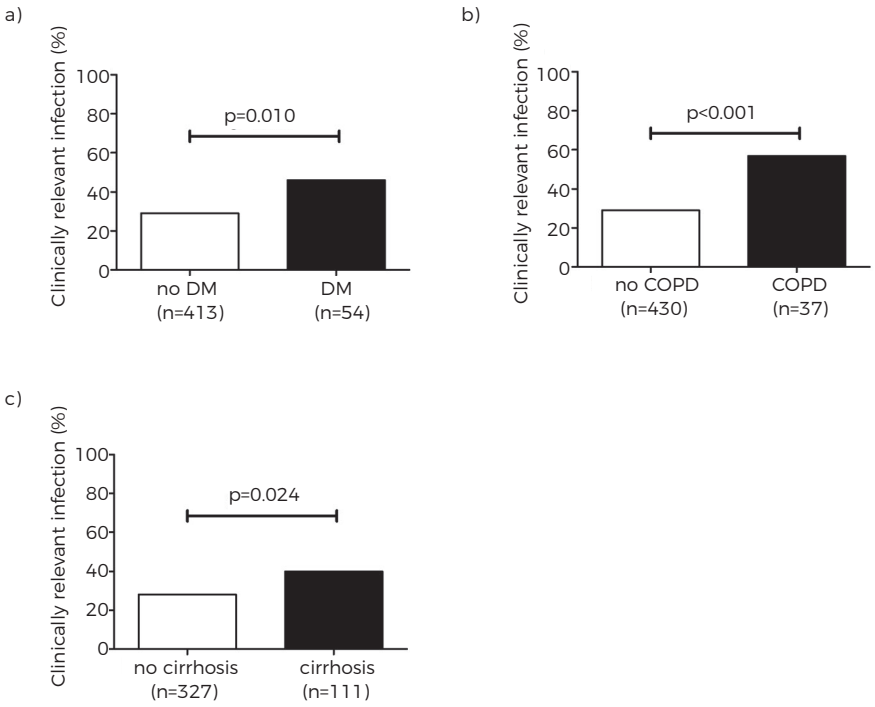
Site of infection	Total infections	Moderate infections	Severe infections
Dermatological	45	40	5
Respiratory	43	29	14
Gastro-intestinal (incl. oral infections)	38	36	2
Renal – Urinary tract	24	22	2
Ear Nose Throat	10	10	0
General	9	4	5
Ophtalmology	5	4	1
Other*	11	6	5
Total	**185**	**151**	**34**

* Other includes: reproductive system (3), musculoskeletal (3), hepatobiliary (2), cardiac or circulatory (2), neutropenic fever.

Table 3. Sites and diagnoses of severe infections

Site of infection	No of infections	Diagnoses
Respiratory	14	Pneumonia (6), pneumonia and exacerbation chronic obstructive pulmonary disease (4), respiratory tract infection (3), viral pleuritis
General	5	Sepsis, febris e causa ignota (3), fever after transfusion of packed cells
Dermatological	5	Abcess, cellulitis, erysipelas, impetigo bacteremia, wound infection after sigmoid resection
Renal – Urinary tract	2	Complicated urinary tract infection, urosepsis
Gastro-intestinal (incl. oral infections)	2	Tooth abscess, gastro-enteritis
Ophtalmology	1	Bacterial eye infection
Other	5	Spontaneous Bacterial Peritonitis (SBP), neutropenic fever, endocarditis, staphylococcal sepsis after phlebitis, osteomyelitis

Figure 2. Clinically relevant infections among patients with known risk factors

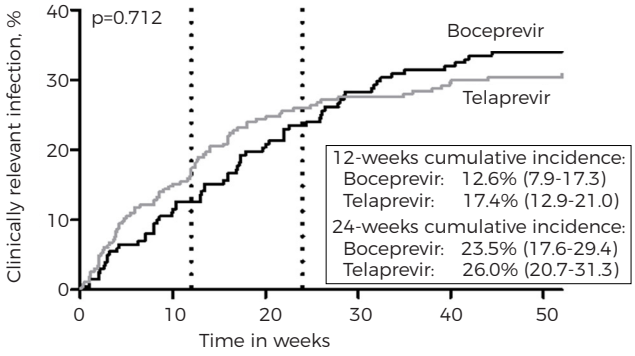


The bars represent the percentage of patients who experienced a clinically relevant infection: 2a) patients with and without diabetes mellitus (DM); 2b) patients with and without chronic obstructive pulmonary disease (COPD); 2c) patients with and without cirrhosis

Risk factors for infection

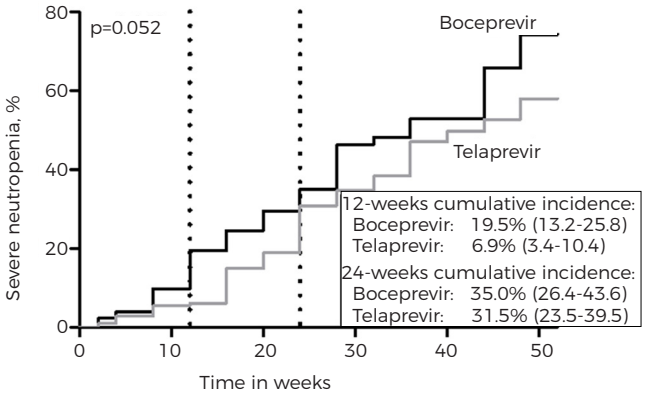
Results of univariable and multivariable logistic regression analysis are shown in Table 4. Neutropenia at the previous visit was not associated with occurrence of infections (univariable OR 0.85, 95%CI 0.57-1.27). Furthermore moderate or severe neutropenia (ANC <750/μL) seemed to be predictive for infection (OR 0.45, 95%CI 0.20-0.99), however this was not significant in the multivariable model. The final multivariable analysis identified female gender (OR 1.7, 95%CI 1.2-2.5), COPD (OR 2.7, 95%CI 1.6-4.5) and presence of DM (OR 1.7, 95%CI 1.0-3.0) as risk factors for infections. The presence of cirrhosis did not reach significance (OR 1.4, 95%CI 0.9-2.1). When adding mild infections to the regression analysis, COPD and female gender remained risk factors, while DM lost significance (p= 0.11)

Figure 3. Cumulative incidence of infections between telaprevir and boceprevir treated patients.



Kaplan-Meier curve showing the occurrence of the first clinically relevant infection during treatment within patients treated with either telaprevir or boceprevir. The 12-weeks and 24-weeks cumulative incidence rates are reported for both telaprevir and boceprevir (with 95% confidence interval).

Figure 4. Time to severe neutropenia between telaprevir and boceprevir treated patients.



Kaplan-Meier curve showing the development of severe neutropenia during treatment within patients treated with either telaprevir or boceprevir. The 12-weeks and 24-weeks cumulative incidence rates are reported for both telaprevir and boceprevir (with 95% confidence interval).

Table 4. Logistic regression analysis with correction for multiple measurements within a patient.

Variable	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Age	1.019 (0.997-1.042)	0.091		
Female sex	1.728 (1.243-2.401)	0.001	1.722 (1.169-2.534)	0.006
Body mass index	0.997 (0.933-1.064)	0.917		
Cirrhosis	1.767 (1.135-2.751)	0.012	1.398 (0.921-2.122)	0.116
Treatment experienced	1.136 (0.758-1.702)	0.537		
Telaprevir vs. boceprevir	1.144 (0.766-1.708)	0.510		
History of decompensated liver disease	1.850 (0.838-4.082)	0.128		
Diabetes mellitus	1.737 (0.959-3.146)	0.069	1.734 (1.008-2.983)	0.047
Chronic Obstructive Pulmonary Disease	3.117 (1.811-5.366)	<0.001	2.701 (1.635-4.463)	<0.001
Use of corticosteroids at baseline	2.312 (1.081-4.946)	0.031		
Leucocyte count per µL	0.995 (0.903-1.096)	0.917		
Neutrophil count per µL	1.066 (0.938-1.211)	0.329		
Absolute neutrophil count < 1500 per µL	0.850 (0.570-1.266)	0.423		
Absolute neutrophil count < 750 per µL	0.448 (0.203-0.989)	0.047		
Baseline albumin, g/L	0.973 (0.925-1.024)	0.293		
Albumin < 35 g/L	1.608 (0.820-3.151)	0.167		
Platelet count x10^9/L	0.996 (0.993-0.999)	0.007		
Platelet count < 100 x10^9/L	1.619 (0.837-3.131)	0.152		
Albumin < 35g/L and platelet count < 100 x10^9/L)	3.091 (0.974-9.813)	0.056		
Bilirubin, g/dL	1.001 (0.999-1.002)	0.388		

OR=Odds Ratio; 95% CI= 95% Confidence Interval

Discussion

Our study demonstrates that a 24-48 week course with boceprevir or telaprevir, pegIFN and RBV for CHC is associated with a high incidence (31%) of clinically relevant infections. Within the first 12 weeks the cumulative incidence of infections was 13-17% depending on the type of protease inhibitor. Skin and respiratory infections were the most commonly seen. The infection incidence rates resemble rates of 12-26% which are reported in literature for pegIFN based therapy with and without a protease inhibitor, indicating the magnitude of this problem.[12-14, 22-24, 27-29] The CUPIC cohort was the first cohort that signaled the high risk for severe infection with first-generation protease inhibitors in cirrhotic patients, and identified two important risk factors: baseline albumin below 35 g/L and baseline platelet count ≤ 100 x10^9/L.[23] Of the cirrhotic patients in our cohort (n=111) 12 patients had both risk factors, and 5 (42%) developed a severe infection, comparable to the CUPIC cohort (51.4%). Presence of only one risk factor led to a severe infection in 22% (albumin < 35 g/L) and 8% (platelets ≤ 100 x10^9/L) of patients with cirrhosis, again resembling CUPIC data.[23] Still, the combined risk factors were not identified as predictor for clinically relevant infections here. The independent factors that drove the risk for infection in our study were female sex, DM and COPD, but not neutropenia. The association of female sex with infections during CHC therapy has been reported previously and was explained by a higher incidence of urinary tract

infections (UTIs) or vaginal infections.[13, 27] Our findings are in agreement with these studies as 14% of clinically relevant infections were UTIs or vaginal infections (n=26) and 92% of these were observed in females. Another explanation might be the higher incidence of cirrhosis in females compared to males in our cohort (54% vs. 22%). Cirrhosis is established as risk factor for infection in literature, whilst our cohort only showed a trend for significance in multivariable analysis.[30-32] The higher proportion of females with cirrhosis in our cohort might have influenced the regression analysis. Diabetes mellitus is a known risk factor for infection, but is also associated with CHC.[33] A higher infection rate in diabetic CHC patients was therefore hypothesized and can be explained by various factors, such as vascular insufficiency and impaired leukocyte function in these patients.[12, 13, 34, 35] Our study implies that diabetic patients should be monitored for infection during CHC therapy. The only other triple therapy cohort study that assessed risk of infection was restricted to cirrhotics and found that respiratory infections were overrepresented in those on protease inhibitor therapy.[22] Our cohort supports this finding, as respiratory infections accounted for 41% of severe infections and 19% of moderate infections. The identified risk factor COPD might relate to this, as COPD is a known risk factor for respiratory infections.[36]
The risk factors in our study (female sex, DM and COPD) are factors that cannot be influenced and are not related to the type of CHC therapy. They are furthermore identified by previous CHC cohorts with (peg)IFN and RBV regimes.[12, 13, 36, 37] It is therefore likely that they remain risk factors for infection in future IFN-containing CHC regimes, thus these patients should be monitored carefully for infection during any pegIFN based regime.

Drug induced neutropenia is thought to be an important risk factor for infection. This stems from oncologic research as development of neutropenia following chemotherapy usually heralds a severe clinical situation necessitating admission and prompt administration of antibiotics.[38] There is a wealth of literature that establishes that pegIFN induced neutropenia does not pose an increased risk for infections in CHC patients.[12, 22, 39] Indeed, in our cohort neutropenia did not increase the risk of infection; it even seemed to be associated with a lower risk for infections. Altogether this suggests that neutropenia due to chemotherapy is different from that due to pegIFN. Oncology patients differ in factors which affect susceptibility for infection such as alteration of organ function caused by their underlying disease and presence of mucosal damage.[40, 41] Because these findings are absent in stable CHC patients, it is reasonable to believe that CHC patients receiving triple therapy are less immune- compromised than oncology patients and that thresholds for pegIFN dose reductions, based on the presence of neutropenia, may be too strict.

The advent of new generation DAAs allows to pinpoint the culprit for neutropenia in CHC. Neutropenia still occurs with any pegIFN containing regimen regardless of DAA included.[42-45] IFN-free regimens do not cause neutropenia suggesting that pegIFN is the cause rather than CHC, DAA or RBV.[43] Here, boceprevir had higher neutropenia

rates than telaprevir. Whether this protease inhibitor interacts with PegIFN for inducing neutropenia or whether it is a class effect of the protease inhibitor cannot be assessed in this study. This finding should be interpreted with caution. Despite the higher incidence of neutropenia, infection rate was comparable between both drugs, confirming the lack of association between neutropenia and infections in CHC therapy.

The strengths of this study are both the size of our real world cohort and its nationwide character. Unique to our cohort is that it includes CHC patients across all fibrosis stages in the Netherlands and is not limited to cirrhotic patients. Patients visited the clinic frequently resulting in detailed records. However, the retrospective design enhances the risk of reporting bias. We made an effort to minimize this risk by adhering to a strict definition of severity of infections and restricting our analysis to infections necessitating anti-infective therapy. Furthermore, telaprevir and boceprevir are first-generation DAAs that have lost market share in view of the advent of more effective and better tolerable new generation DAAs. However these drugs continue to be used in economically deprived countries that use pegIFN as a backbone for CHC therapy.[46, 47]

Conclusion

Our real world nationwide cohort study showed that the incidence of infections during pegIFN-based triple therapy is high, even among patients without cirrhosis. Neutropenia occurs frequently, but does not increase the risk for infection. Female gender, DM and COPD however, were risk factors for infection and are independent of type of CHC therapy, suggesting that these patients should be carefully monitored for infections once a pegIFN-based regimen is initiated.

Acknowledgements

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Abbreviations

ANC	Absolute Neutrophil Count
CHC	Chronic Hepatitis C
COPD	Chronic Obstructive Pulmonary Disease
DAAs	Direct-Acting Antivirals
DM	Diabetes Mellitus
HCV	Hepatitis C Virus
OR	Odds Ratio
pegIFN	Pegylated interferon-α
95%CI	95% Confidence Interval

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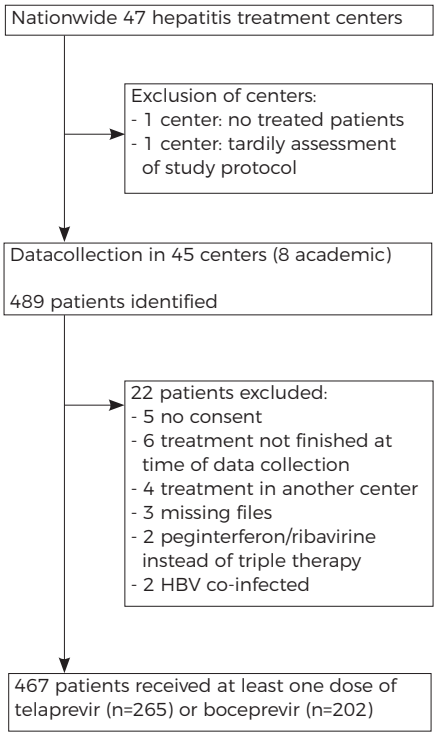
Supplementary File

Supplementary Figure 1. Study flowchart




4

Bleeding episodes during interferon-based triple therapy in hepatitis C: a nationwide cohort study

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Submitted.

Abstract

Background

In many countries, treatment of chronic hepatitis C (CHC) continues to be based on pegylated interferon (pegIFN) and ribavirin, sometimes with addition of a protease inhibitor (PI). Thrombocytopenia is an important side effect of pegIFN and may lead to dose reductions out of fear for bleeding episodes. The primary aim of this study is to assess the incidence and risk factors for bleeding episodes in patients on first-generation PI based therapy.

Methods

We established a nationwide retrospective cohort study of CHC patients who were treated with a first-generation PI in addition to pegIFN and ribavirin. We evaluated the cumulative incidence of bleeding episodes and thrombocytopenia (platelet count <150 $x10^9$/L). Risk factors for bleeding episodes were identified through multivariable logistic regression analysis, adjusting for multiple measurements within a patient.

Results

This cohort contains 467 CHC patients, 319 (68%) male patients and 111 (25%) cirrhotics. Baseline thrombocytopenia was present in 139 (31%) patients and 379 (81%) patients experienced on-treatment thrombocytopenia (73 patients with platelet count <50 $x10^9$/L). Overall, 103 patients reported a bleeding episode which were mainly mild (topical or no treatment given). Cirrhosis (OR 2.2, 95%CI 1.4-3.5), platelet count below 50 $x10^9$/L at the previous visit (OR 2.1, 95%CI 1.3-3.5) and female sex (OR 1.6, 95%CI 1.1-2.5) were associated with a bleeding episode.

Conclusions

Risk factors for on-treatment bleeding episodes were female sex, presence of cirrhosis and a platelet count below 50 $x10^9$/L at the previous visit. PegIFN dose reductions during triple therapy should be considered thoroughly, as the majority of bleeding episodes were mild.

Introduction

The development of direct-acting antivirals (DAAs) has revolutionized the treatment of chronic hepatitis C virus (CHC) infection. Peginterferon (pegIFN)-free and even ribavirin (RBV)-free regimens result in high rates of sustained virological response (SVR) while having excellent safety profiles.[1, 2] The only factor that hampers global access to these drugs is pricing.[3] Policy makers are forced to prioritize treatment to those patients who are considered to have an urgent need for successful antiviral therapy.[4] In many countries, treatment of CHC infection still involves the combination of pegIFN and RBV, sometimes with the addition of a protease inhibitor (PI).[5-8]
Unfortunately, therapy with pegIFN and RBV is associated with many side effects, including haematological disorders. One of the major side effects is the development or aggravation of thrombocytopenia which is thought to contribute to the risk for bleeding episodes.[9-11] Guidelines and the label advise to reduce the dose of pegIFN in case platelet count falls below 50 $x10^9$/L and to stop treatment when below 25 $x10^9$/L.[4,12] Therefore, onset of thrombocytopenia usually prompts pegIFN dose reductions curtailing its treatment efficacy for dual therapy with pegIFN and RBV.[13-15] Some studies question this dose-adaption strategy as major bleeding episodes have not occurred in a high frequency during pegIFN-based therapy.[16-19] There are a number of reasons to revisit this issue. First, real-world studies have demonstrated that telaprevir or boceprevir based regimens come with high rates of adverse events, particularly among patients with cirrhosis.[20-22] Moreover, a recent meta-analysis found that PI-based therapy amplifies the occurrence of thrombocytopenia.[23] Whether triple therapy with telaprevir or boceprevir amplifies the risk for bleeding episodes is unknown. Therefore, the aims of this study were (i) to assess the incidence and risk factors for bleeding episodes during first-generation PI-based triple therapy, and (ii) to assess the influence of on-treatment thrombocytopenia on bleeding episodes.

Methods

Population and design

This nationwide cohort study includes patients with CHC genotype 1 treated between 2011 and 2015 with first-generation PIs in the Netherlands.[24] Patients were identified from local databases in 45 hospitals, and those co-infected with hepatitis B virus or human immunodeficiency virus were excluded. Due to the retrospective character of the study, formal approval was waived by the institute review board Arnhem-Nijmegen. Local regulations were followed per participating centre. The study was conducted following good clinical practice guidelines and the code of conduct (www.federa.org).

Outcomes and definitions

The primary outcome of this study is the occurrence of bleeding episodes from start of therapy until 4 weeks after cessation of therapy. Secondary outcomes include: severity of bleeding episodes, occurrence and severity of thrombocytopenia, and risk factors for bleeding episodes (including on-treatment thrombocytopenia). We assessed time to the first bleeding episode and time to severe thrombocytopenia as current pegIFN containing regimes with DAAs allow shortened treatment durations.[25] Bleeding episodes were defined as severe in case of death, hospital admission or transfusion of packed cells or thrombocytes; moderate when bleeding led to an intervention; and mild if topical or no treatment was required. Likewise, thrombocytopenia was categorized as severe if platelet count was below 50 $x10^9$/L, moderate if platelet count was between 50 and 74 $x10^9$/L, and mild if platelet count was between 75 and 149 $x10^9$/L.[16, 17, 26] Conventional fibrosis assessment (liver biopsy or transient elastography) was missing in 159 patients (34%), and therefore Fib-4 index > 3.25 was used to diagnose cirrhosis.[27] History of decompensated liver disease was defined as a history of ascites, hepatic encephalopathy, or variceal bleeding. We categorized bleeding sites by common terminology criteria for adverse events.[28]

Data acquisition

Data was collected from the patients medical records. We extracted demographic data, disease characteristics, bleeding episodes and laboratory values during and after treatment. When two bleeding episodes were reported between two visits, the most severe bleeding episode was included. Concomitant mediation at baseline affecting the risk of bleeding (vitamin K antagonists, heparin, thrombocyte aggregation inhibitors, vitamin K, and coagulation factors) was collected as well.

Statistical analysis

Variables were presented as mean, median or proportion depending on variable type. Differences between patients with and without cirrhosis, and with telaprevir vs. boceprevir treatment were assessed using Chi-square test. To identify risk factors for bleeding episodes, logistic regression analyses with correction for multiple measurements within one patient were performed. Age, sex, cirrhosis and variables with $p \leq 0.2$ in univariable analysis were included in the multivariable analysis. Time to the first bleeding episode and severe thrombocytopenia were assessed using the Kaplan Meier method. Tests were two-sided with a significance level of $p < 0.05$. The analyses were performed using SPSS (IBM SPSS Statistics 20) and SAS 9.3 (SAS Institute, Cary, NC, USA).

Results

Population

In total, 489 CHC patients were included and 22 were excluded (Supplementary Figure 1). Our cohort comprises 467 patients: 265 were treated with telaprevir and 202 with boceprevir based regimens (Table 1). It consists of 319 (68%) males and mean age was 50.8 years (SD 9.6). The cohort included 111 (25%) patients with cirrhosis and 23 (5%) patients with hemophilia (9 classified as severe hemophilia with factor activity <1%).

Table 1. Baseline characteristics of patients with and without a bleeding episode

Characteristic	Overall (n= 467)	Patients without bleeding (n= 364)	Patients with bleeding (n= 103)
Age in years – mean (range)	51 (19-77)	51 (21-77)	51 (19-69)
Male sex – n (%)	319 (68)	262 (72)	57 (55)
White race – n (%)[a]	321 (89)	244 (90)	77 (88)
Fib 4 index – median (IQR)[b]	1.8 (1.1-3.3)	1.6 (1.1-2.9)	2.3 (1.3-6.8)
Fib 4 index > 3.25 – n (%)[b]	111 (25)	74 (22)	37 (39)
Decompensated liver disease – n (%)	24 (5)	16 (4)	8 (8)
Hemophilia – n (%)	23 (5)	17 (5)	6 (6)
Telaprevir vs. boceprevir – n	265 vs. 202	200 vs. 164	65 vs. 38
Baseline concomitant medication			
Use of anticoagulants – n (%)	32 (7)	25 (7)	7 (7)
Use of hemostatics – n (%)	22 (5)	15 (4)	7 (7)
Laboratory values[c]			
Hemoglobin g/dl – mean (SD)	9.1 (0.9)	9.1 (0.9)	9.2 (0.8)
Leucocyte count/µL – mean (SD)	6727 (2154)	6770 (2190)	6571 (2022)
Neutrophil count/µL – mean (SD)	3454 (1533)	3508 (1513)	3283 (1590)
Platelet count x10^9/L – mean (SD)	192 (76)	196 (78)	178 (67)
Thrombocytopenia at baseline (platelet count <150 x10^9/L) – n (%)[d]	139 (31)	103 (30)	36 (37)
Albumin g/L – mean (range)	41 (24-51)	41 (24-51)	42 (27-50)

[a] Race: available for 360 patients; [b] Fib-4 score score: available for 438 patients; [c] Lab values >10% missings in: neutrophil count and albumin; [d] Platelet count at baseline: available for 446 patients

Bleeding episodes

A total of 130 bleeding episodes were reported in 103 (22%) patients. Of the 130 episodes, 13 (10%) were classified as moderate and 10 (8%) as severe. Two patients, a 63 year old male and 48 year old female, died following a hemorrhagic cerebrovascular accident (CVA). Both patients had cirrhosis and platelet count prior to the CVAs were 76 x10^9/L and 35 x10^9/L, respectively. A total of 6 patients were 8 times admitted to the hospital due to a bleeding event (Table 2). Most frequent sites of bleeding events in general were gastro-intestinal (n=49), ear-nose-throat (n=48), and skin (n=21). The median time to the first bleeding episode was 8 weeks (IQR 4-16).

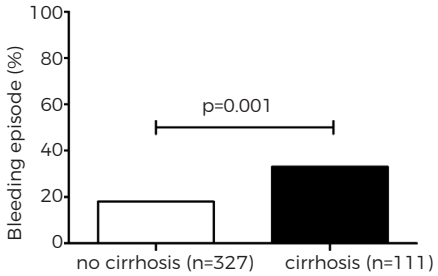
A higher proportion of patients with cirrhosis experienced at least one bleeding episode compared to patients without cirrhosis (33% vs. 18%, p=0.001; Figure 1). Multiple bleeding episodes (range 2-4) occurred in 19 patients. The total amount of bleeding episodes was numerically higher when telaprevir was a component of antiviral therapy, however non-significant (p=0.768, Figure 2). Cirrhotic patients treated with telaprevir had a higher cumulative incidence of bleeding episodes compared to boceprevir patients (p=0.029), this PI dependent difference was absent in non-cirrhotic patients (p=0.337). The 12-week and 24-week cumulative incidence of bleeding episodes among cirrhotic patients treated with telaprevir were 27.4% (95%CI 16.2-38.6) and 38.7% (95%CI 26.5-50.9) respectively. Among the cirrhotic patients treated with boceprevir the 12-week and 24-week cumulative incidence of bleeding episodes were 12.2% (95%CI 3.0-21.4) and 18.4% (95%CI 7.6-29.2) respectively. Among cirrhotic female patients the 12-week and 24-week cumulative incidence of bleeding episodes were 25.0% (95%CI 13.2-36.8) and 38.5% (95%CI 25.4-51.6) respectively. The 12-week and 24-week cumulative incidence of bleeding episodes among cirrhotic male patients were 16.9% (95%CI 7.3-26.5) and 22.0% (95%CI 11.4-32.6) respectively (Figure 3).

Table 2. Sites and severity of bleeding episodes

Bleeding site	Total	Mild	Moderate	Severe[e]
Gastrointestinal[a]	49	39	6	4
Ear Nose Throat[b]	48	42	3	3
Skin[c]	21	21	0	0
Respiratory[d]	4	2	2	0
Reproductive system	4	2	2	0
Central Nervous System	3	0	0	3
Renal	1	1	0	0
Total no of bleedings	**130**	**107**	**13**	**10**

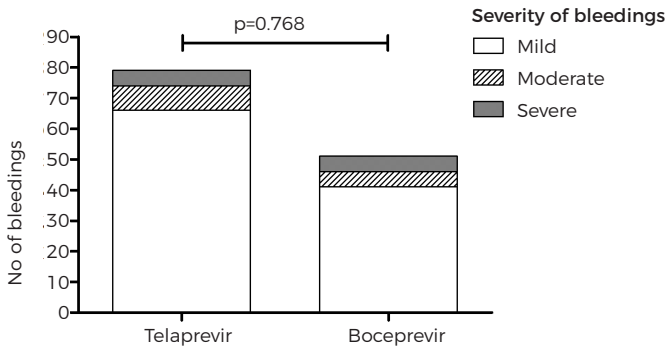
[a] mainly rectal blood loss (n= 33) and gingival bleeding (n= 10) [b] all epistaxis (n= 48) [c] mainly hematomas (n= 20) [d] all hemoptoe (n=4) [e] Severe bleeding episodes: hematemesis (3), rectal blood loss (1), epistaxis (3), hemorrhagic cerebrovascular accident (2), subdural hematoma (1)

Figure 1. Occurrence of bleeding episodes according to the presence or absence of cirrhosis



The bars represent the proportion of patients who reported at least one bleeding episode according to the presence or absence of cirrhosis . In cirrhotic patients 37 (33%) of 111 reported a bleeding episode, while 59 (18%) of 327 patients without cirrhosis reported a bleeding episode. For 29 patients cirrhotic status was missing.

Figure 2. Total amount and severity of bleeding episodes per protease inhibitor



The bars represent the number of bleeding events and the filling differentiates severity of these events. In total 79 (66 mild, 8 moderate, 5 severe) bleeding episodes were reported among 65 patients treated with telaprevir and 51 (41 mild, 5 moderate, 5 severe) among 38 patients treated with boceprevir.

Risk factors for bleeding episodes

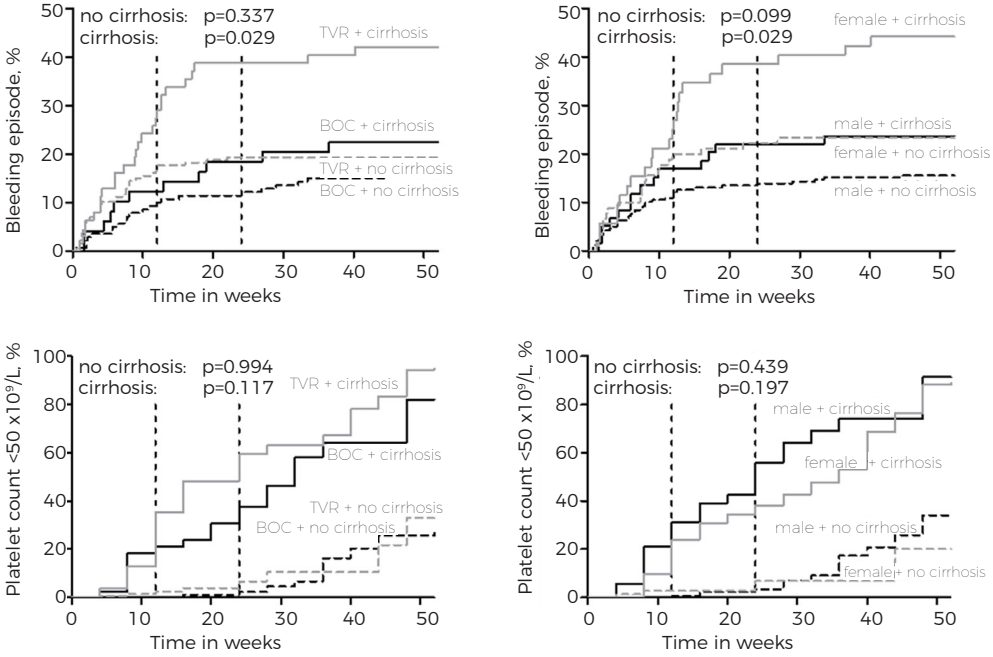
Multivariable regression analysis revealed three independent risk factors for on-treatment bleeding episodes (Table 3): presence of cirrhosis (odds ratio (OR) 2.2, 95% confidence interval (CI) 1.4-3.5), severe thrombocytopenia at the previous visit (OR 2.1, 95%CI 1.3-3.5) and female sex (OR 1.6, 95%CI 1.1-2.5). Hemophilia, type of PI or use of anticoagulant medication were not associated with the risk for bleeding episodes.

Thrombocytopenia and bleeding episodes

In our cohort, baseline thrombocytopenia was present in 139 (31%) patients (7 moderate, 4 severe). Fifty-four (39%) of these patients developed severe thrombocytopenia in addition to the 4 patients who had severe thrombocytopenia at baseline. Only one of these 4 patients accomplished the therapy of 48 weeks, the others discontinued treatment early at week 3, 4 and 37 respectively, and none of them reported a bleeding episode. From the patients with normal or missing platelet count at baseline (n=307 and n=21), 240 developed thrombocytopenia. Thus, overall 379 patients (81%) experienced on-treatment thrombocytopenia (moderate n=80, severe n=73). This was independent of telaprevir or boceprevir use (Figure 3). Median drop of thrombocyte count was 81 $x10^9$/L (IQR 56-116 $x10^9$/L), and median time to reach nadir platelet count was 12 weeks (IQR 8-24). Platelet count was available for 4719 (95%) visits, and laboratory results were consistent with thrombocytopenia in 2847 (60%) of those visits (300 visits severe thrombocytopenia). After 89 (3%) of the 2847 visits with thrombocytopenia, a bleeding episode was reported at the subsequent visit, meaning that 2758 (97%) of the visits were not followed by a reported bleeding event. Moreover, 24 (8%) of the 300 visits with severe thrombocytopenia were followed by a reported bleeding episode. In addition,

40 bleeding events were reported with a normal platelet count at the preceding visit. Median platelet count at the visit prior to a bleeding episode was 98 x10^9/L (IQR 56-163 x10^9/L).

Figure 3. Cumulative incidence of bleeding episodes and severe thrombocytopenia



Kaplan-Meier curves showing the occurrence of bleeding episodes (panel a and b) and severe thrombocytopenia (panel c and d). The dashed vertical lines represent the 12-week and 24-week time points. Panel a and b demonstrate the time to the first bleeding episode according to the cirrhotic status for the use of telaprevir or boceprevir (TVR, BOC, a) and for males and females (b). Panel c and d demonstrate the occurrence of severe thrombocytopenia for patients treated with TVR and BOC (c) and for males and females (d), based on the presence and absence of cirrhosis. The table below shows the 12-week and 24-week cumulative incidences of the first bleeding episode and severe thrombocytopenia for each subgroup (presented as % (95%CI).

		First bleeding episode		Severe thrombocytopenia	
		12-week cumulative incidence	24-week cumulative incidence	12-week cumulative incidence	24-week cumulative incidence
No cirrhosis	Boceprevir	9.3 (4.4-14.2)	11.4 (6.1-16.7)	no events	1.0 (0.0-3.0)
	Telaprevir	16.6 (11.3-21.9)	19.3 (13.6-25.0)	1.3 (0.1-3.1)	3.9 (0.0-8.0)
	Male	11.0 (7.1-14.9)	13.5 (9.2-17.8)	no events	2.2 (0.0-4.7)
	Female	20.0 (11.8-28.2)	22.2 (13.6-30.8)	2.7 (0.0-6.4)	2.7 (0.0-6.4)
Cirrhosis	Boceprevir	12.2 (3.0-21.4)	18.4 (7.6-29.2)	20.2 (8.4-32.0)	31.9 (17.2-46.6)
	Telaprevir	27.4 (16.2-38.6)	38.7 (26.5-50.9)	12.8 (3.2-22.4)	48.3 (31.6-65.0)
	Male	16.9 (7.3-26.5)	22.0 (11.4-32.6)	22.4 (10.6-34.2)	43.5 (28.2-58.8)
	Female	25.0 (13.2-36.8)	38.5 (25.4-51.6)	9.8 (0.8-18.8)	34.4 (18.1-50.7)

Table 3. Regression analysis with correction for multiple measurements within a patient.

Variable	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Age	1.004 (0.986-1.023)	0.651		
Female gender	1.987 (1.326-2.977)	<0.001	1.632 (1.060-2.513)	0.026
Body mass index	0.957 (0.739-1.240)	0.741		
Hemophilia	1.144 (0.527-2.485)	0.734		
Diabetes Mellitus	0.648 (0.319-1.313)	0.228		
Cirrhosis	2.909 (1.903-4.446)	<0.001	2.181 (1.356-3.508)	0.001
History of decompensated liver disease	1.494 (0.704-3.167)	0.296		
Use of anticoagulants	1.268 (0.409-3.927)	0.681		
Use of hemostatics	1.855 (0.854-4.030)	0.119		
Treatment experienced	0.720 (0.465-1.116)	0.142		
Telaprevir vs. boceprevir	1.369 (0.860-2.178)	0.186		
Platelets	0.995 (0.992-0.998)	0.002		
Platelet count < 100 x10^9/L at previous visit	1.721 (0.875-3.382)	0.116		
Platelet count < 50 x10^9/L at previous visit	3.486 (2.213-5.491)	<0.001	2.097 (1.257-3.498)	0.005
Baseline albumin	1.000 (0.999-1.001)	0.419		
Albumin < 35 g/L	2.101 (1.044-4.227)	0.038		
Albumin < 35g/L and platelet count < 100 x10^9/L)	1.673 (0.545-5.139)	0.369		
Bilirubin	1.000 (0.999-1.001)	0.788		

OR: Odds Ratio; 95%CI: 95% confidence interval

Discussion

One of our key findings is that ~20% of patients experienced at least one bleeding episode during 24-48 weeks of triple therapy with a first-generation PI, pegIFN and RBV. The majority of these bleeding episodes were mild and required no or only topical treatment. Overall cumulative incidence of bleeding episodes was 15% (95%CI 12-19) at 12 weeks and 19% (95%CI 16-23) at 24 weeks of therapy. We found that the presence of cirrhosis, severe on-treatment thrombocytopenia at the previous visit, and female sex were associated with an increased risk for a bleeding event during therapy.

The proportion of patients experiencing a bleeding episode in our study (~20%) was higher than reported with pegIFN and RBV dual therapy (8-10%). The difference is that these cohorts included only patients with advanced fibrosis, while sex distribution and incidence of thrombocytopenia were comparable to our cohort.[16, 17, 19] The addition of a PI in our cohort might be an explanation for the higher incidence of bleeding episodes.

Cirrhosis was an independent risk factor for bleeding episodes in our cohort with patients of all fibrosis stages. These results are in line with findings from (peg)IFN with or without RBV treated cohorts.[16, 17] Previous studies attempted to explain the balance of bleeding risk and hypercoagulation in cirrhotic patients. It is thought that thrombocytopenia, defective platelet aggregation but also hemodynamic alterations, endothelial dysfunction, bacterial infections and renal failure contribute to bleeding tendency in cirrhotics.[29, 30] Severe thrombocytopenia was a second predictor of bleeding

episodes in our cohort and was experienced by 73 (16%) patients. Thrombocytopenia can be caused by the hepatitis C virus, liver cirrhosis and pegIFN. Suggested mechanisms involved are: decreased thrombopoietin activity, immune dysfunction, bone marrow suppression by the hepatitis C virus or pegIFN, and splenic platelet sequestration in case of portal hypertension.[10, 31, 32] Female gender was the third identified risk factor. An increased incidence of bleeding episodes in females is not only observed during CHC therapy but is reported more frequently in literature, for example after invasive cardiovascular procedures.[16, 33, 34] An extensive review has investigated sex differences and noted that bleeding time is increased in females, possibly due to sex hormone induced altered platelet activity.[35]

It is thought that pegIFN is the most likely agent to cause thrombocytopenia or aggravation of thrombocytopenia due to myelosuppression.[10, 36] Incidence rates up to 48% are reported in literature.[19, 37, 38] Current available pegIFN free regimens support this hypothesis as therapy induced thrombocytopenia has not been reported by several landmark studies, while pegIFN containing regimens with new generation DAAs still report thrombocytopenia.[39, 40] Dose reductions of pegIFN are recommended to prevent bleeding episodes, but this curtails effectiveness.[13-15] Other options to improve platelet count are treatment with thrombopoietin receptor agonists, such as eltrombopag or avatrombopag, however their use is limited due to the risk of hepatic decompensation and tromboembolic events.[41, 42]

We specifically assessed a cohort treated with two different PIs added to pegIFN and RBV and assessed the influence of the drugs on thrombocytopenia and bleeding events. Whereas there was no difference in cumulative incidence of severe thrombocytopenia between both drugs, we found higher proportion of bleeding episodes in telaprevir treated cirrhotic patients. There were no differences in patient characteristics and particularly sex, cirrhosis and platelet counts were comparable between patients treated with telaprevir or boceprevir. The literature indicates that the pharmacokinetics of telaprevir is independent from presence of cirrhosis.[43] Hence, it is possible that the risk for bleeding is a class effect of PIs. Future studies in cirrhotic patients with new generation PIs, such as simeprevir, grazoprevir or paritaprevir may provide evidence whether there is a synergistic effect of some PIs to pegIFN or whether the effect is PI specific.

Our study comes with some strengths and limitations. Strengths are the size of the cohort with patients across all fibrosis stages, and the high number of visits resulting in detailed data within a long timeframe. The nationwide character with both academic and regional hospitals results in a representative cohort of patients. Limitations include the retrospective design which can lead to reporting bias. By defining bleeding episodes carefully we sought to reduce this bias and probably only mild bleeding episodes were missed. Further, although pegIFN-free regimens are available, many countries still use pegIFN as backbone hence our results remain applicable to clinical practice.[8, 44]

Conclusions

Bleeding episodes occurred frequently (22%) among patients in this real world CHC cohort who were treated with triple therapy. Female sex, the presence of cirrhosis and platelet count below 50 x10^9/L were associated with an increased risk for bleeding episodes, which were however mostly mild. PegIFN dose reductions during triple therapy should be considered thoroughly.

Acknowledgements

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Abbreviations

CHC	Chronic Hepatitis C
CVA	CerebroVascular Accident
DAAs	Direct Acting Antivirals
IFN	Interferon-α
IQR	Interquartile range
OR	Odds Ratio
pegIFN	Pegylated interferon-α
PI	Protease Inhibitor
RBV	Ribavirin
SVR	Sustained Virological Response
95%CI	95% Confidence Interval

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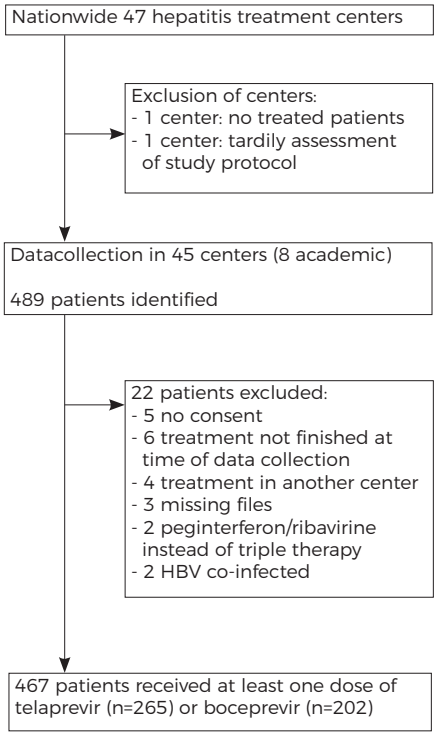
Supplementary File

Supplementary Figure 1. Study flowchart



This supplementary figure was published before (Berden, F; van Zwietering, I; Maan, R; de Knegt R; Kievit, W; Drenth, J; High Risk of Infection During Triple Therapy with First-Generation Protease Inhibitors: A Nationwide Cohort Study. J Gastrointestin Liver Dis, June 2016 Vol. 25 No 2.)

The gap between registration trials and real world in hepatitis C is closing

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To the editor:

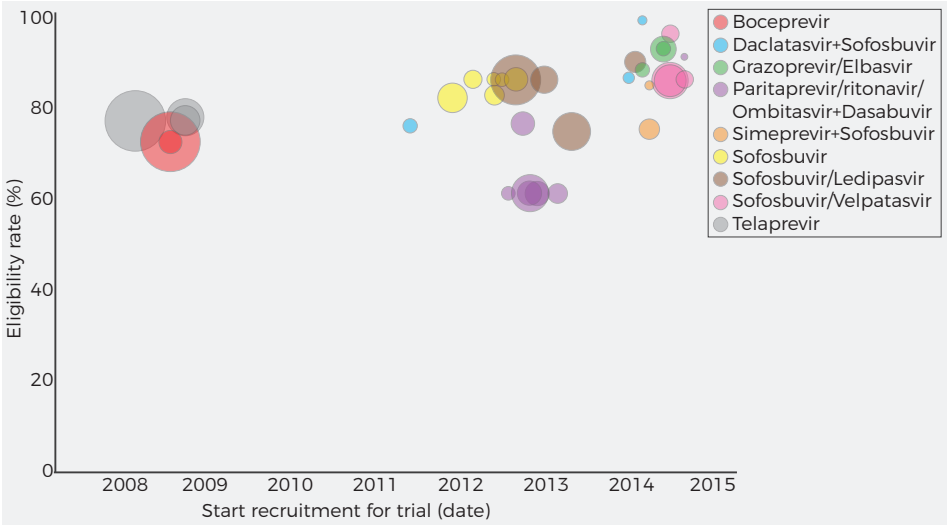
The development of direct acting antivirals (DAAs) for chronic hepatitis C (HCV) infection was supported by many registration trials. However, trial patients appeared to be different from real world patients, and this puts limits on generalization. The first-generation DAAs, while effective and safe in trials, were less effective and more toxic in HCV patients with advanced liver disease.[1] Previously, we suggested that strict eligibility criteria of trials contributed to the gap between trials and practice.[2] The newer DAA regimens are highly effective and come with a better safety profile. We hypothesize that eligibility criteria of DAA registration trials have become more lenient over time, contributing to a better generalizability.[3]

To assess the eligibility rate (proportion of patients fulfilling inclusion and exclusion criteria) over time, we checked eligibility criteria of DAA registration trials against the profile of a real world HCV cohort (n=177). We identified 43 registration trials of approved DAAs based on the labels of U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA), and we were able to access full eligibility criteria of 34 trials. Eligibility per patient was determined on the thresholds of seven criteria per trial: decompensated liver disease, cirrhosis, bilirubin, albumin, hemoglobin, platelet and neutrophil count. These factors were repeatedly identified as predictors for response in real world cohorts with DAA regimens, indicating that these criteria are indeed relevant to generalizability of outcomes.[2, 4]

In Figure 1 we plotted the eligibility rate of 34 registration trials with nine DAA regimens against the start date of inclusion. Some points merit attention. First, the eligibility rate of trials has improved over time. Linear regression analysis demonstrates a positive association between eligibility rate and time in months (intercept=70.9; slope=0.18, p=0.02). Second, in some regimens (e.g. sofosbuvir/daclatasvir, blue circles in Figure 1) we saw that later trials had less stringent inclusion criteria than trials that commenced recruiting earlier. Although the exact reasons for lowering the threshold are unknown, we believe this might depend on factors such as progressing safety knowledge, market opportunities, requests from the field or authorities. Third, the circle size is decreasing over time, indicating that trials that started later, had fewer patients. Lastly, highest eligibility rates (96-99%) were seen in trials allowing decompensated cirrhotics (ALLY-1/ASTRAL-4). Our findings are corroborated by real world cohorts that report similar effectiveness as registration trials.[4, 5]

In conclusion, the eligibility criteria of registration trials testing DAAs for HCV became less strict over time. This has closed the once existing gap between trials and real world. Registration trials should cover the complete phenotypical spectrum of a disorder to result in high eligibility rates. Predictors of response are related to the eligibility criteria affecting outcomes, thus mainly these criteria should be lenient.

Figure 1. Eligibility rate of hepatitis C patients for DAA trials over time



This figure shows that the proportion of real world patients who are eligible for registration trials (y-axis) testing DAAs (colors) improved over time (x-axis). The size of the circles represents the number of patients per registration trial.

Abbreviations

DAAs	Direct-acting Antivirals
HCV	Hepatitis C Virus

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6

The majority of hepatitis C patients treated with direct-acting antivirals are at risk for relevant drug-drug interactions

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Abstract

Background
Direct-acting antivirals have improved treatment of chronic hepatitis C virus infection significantly. Direct-acting antivirals inhibit/induce and can also be substrates of, drug-metabolizing enzymes and transporters. This increases the risk for drug-drug interactions.

Objective
The purpose of this study was to predict drug-drug interactions with co-medication used by hepatitis C virus-infected patients.

Methods
We assembled a nationwide cohort of hepatitis C patients and collected cross-sectional data on co-medication use. We compiled a list of currently available direct-acting antiviral regimens and cross-checked for potential drug-drug interactions with used co-medication.

Results
The cohort included 461 patients of which 77% used co-medication. We identified 260 drugs used as co-medication. Antidepressants (7.4%), proton pump inhibitors (7.1%), and benzodiazepines (7.1%) were most frequently used. Of the patients, 60% were at risk for a clinically relevant drug-drug interaction with at least one of the direct-acting antivirals regimens. Interactions were most common with paritaprevir/ritonavir/ombitasvir/dasabuvir and least interactions were predicted with grazoprevir/elbasvir.

Conclusion
Co-medication use is rich in frequency and diversity in chronic hepatitis C patients. The majority of patients are at risk for drug-drug interactions which may affect efficacy or toxicity of direct-acting antivirals or co-medication. The most recently introduced direct-acting antivirals are associated with a lower risk of drug-drug interactions.

Introduction

Treatment of hepatitis C virus (HCV) infected patients has significantly improved with the introduction of direct-acting antivirals (DAAs). DAAs have the disadvantage that they can be involved in drug-drug interactions (DDIs) with patients' co-medication. DDIs might increase the risk for toxicity or result in poorer efficacy.[1, 2] The mechanism is twofold: DAAs can both be victim and/or perpetrator of DDIs. Drugs are victims of DDIs when their plasma concentration is affected by another drug. In contrast, drugs are perpetrators when they have the ability to influence plasma concentrations of drugs, for example by inhibiting or inducing metabolizing enzymes and/or drug- transporters.[3, 4] DAAs inhibit various cytochrome P450 (CYP) enzymes, responsible for drug metabolism (Table 1). The clinical importance of DDIs was illustrated by the interaction between sofosbuvir-based DAA therapy and amiodarone, resulting in severe bradycardia.[5, 6] This report and other similar papers indicate that there is a genuine risk for relevant DDIs in patients treated with DAAs who use co-medication.[5-11]

The toxicity profiles of the currently used interferon-free DAA combinations, improved significantly relative to the DAAs combined with peginterferon and ribavirin. Nowadays, more HCV patients with complex co-morbidities and thus co-medication receive antiviral treatment.[12] The combination of DAAs and many other drugs obviously increases the risk for DDIs. To date, limited data is available about the extent of co-medication use by HCV patients and the risk of DDIs as a consequence. Therefore, the aim of this study is to identify the co-medication use in a nationwide real-life HCV cohort in order to predict clinically relevant DDIs between co-medication and new DAA regimens.

Table 1. Overview of enzymes and drug transporters involved in the metabolism and transport of DAAs used for the treatment of hepatitis C.

Direct-acting Antiviral	Victim (= substrate of)	Perpetrator *Inhibitor*	*Inducer*
Daclatasvir	CYP3A4/5, P-gp	P-gp, OATP1B1	
Dasabuvir	CYP2C8, CYP3A4, P-gp, BCRP	UGT1A1, BCRP, P-gp	
Elbasvir	CYP3A, P-gp	-	
Grazoprevir	CYP3A, P-gp, OATP1B1/3	CYP3A (?)	
Ledipasvir	Pg-p, BCRP	P-gp, BCRP	
Ombitasvir	-	UGT1A1	
Paritaprevir/ritonavir	CYP3A4/5, P-gp, OATP1B1/3, BCRP	CYP3A4/5, UGT1A1, CYP2D6(?),OATP1B1/3, OATP2B1, BCRP	CYP2C19
Simeprevir	CYP3A4/5	CYP3A4/5, CYP1A2, P- gp, OATP1B1/3	
Sofosbuvir	P-gp, BCRP	-	
Velpatasvir	P-gp, BCRP, CYP2B6, CYP2C8, CYP3A4	P-gp, BCRP, OATP1B1/2, OATP2B1	

?: Unknown if the inhibition/induction is clinically relevant. DAA: Direct Acting Antiviral, CYP: cytochrome P450, BCRP: breast cancer resistance protein , P-gp: P-glycoprotein , UGT: uridine diphosphate glucuronosyltransferase , OATP: organic anion-transporting polypeptide

Methods

We performed this research in three steps: (a) we identified which co-medication were used by HCV-infected patients in a real-world cohort; (b) in order to predict DDIs we cross-checked the co-medication with DAAs in the database of the University of Liverpool (www.hep-druginteractions.org); and (c) we assessed the risk for DDIs per patient. For this type of study (retrospective) formal consent was not required. Formal evaluation was waived by the institutional review board Arnhem-Nijmegen. Good Clinical Practice guidelines and the code of conduct for the use of data in health research were followed (www.federa.org).

Patients and use of co-medication

Data from a nationwide, real-life cohort were used.[13] This cohort included Dutch patients treated for an HCV genotype 1 mono-infection. Patients were identified based on local databases present in 45 hepatitis treatment centers in the Netherlands. Data collection was performed between January 2014 – July 2015. Baseline data were extracted from the patient's medical record and included patient characteristics, medical history, HCV genotype and co-medication use prior to commencement of HCV treatment. Patients were excluded when data on co-medication use was missing and if patients had a co-infection with HIV or hepatitis B virus. In addition to prescribed medication, we included complementary and alternative medicine (CAM) when available in the medical record. Separate compounds of fixed-dose products were registered, except for CAMs, these were counted as one, even though they may have contained several chemical compounds. We did include drugs taken as part of a substance abuse disorder (e.g. methadone), although illicit drugs such as heroin or cocaine were not collected. We added Anatomical Therapeutic Chemical (ATC) codes to all co-medication reported in the patient's medical record, and grouped the drugs by therapeutic/pharmacological subgroups.[14]

Predicted drug-drug interactions with DAAs

The co-medication was cross-checked with currently approved DAA regimens in Europe and USA through the University of Liverpool database in an effort to predict DDIs (July 2016). The University of Liverpool database is a commonly used resource to check for DDIs.[4, 15] For cross-checking we included approved DAA regimens effective against HCV genotype 1: sofosbuvir plus simeprevir, sofosbuvir plus daclatasvir, sofosbuvir plus ledipasvir, paritaprevir/ritonavir, ombitasvir plus dasabuvir, elbasvir plus grazoprevir, and sofosbuvir plus velpatasvir. Ribavirin and first-generation protease inhibitors were not taken into account. Ribavirin is considered not to cause any DDIs in this population as is not metabolized by or influencing any of the drug metabolizing enzymes and the included patients do not use nucleoside reverse transcriptase inhibitors (NRTIs).[16] The first-generation DAAs are considered outdated.

We used four risk categories corresponding with the University of Liverpool database: 1) No clinically significant interaction; 2) Potential interaction - may require close monitoring, alternation of drug dosage or timing of administration; 3) Contraindication, i.e. drugs should not be co-administered; 4) Unknown, as not available in the Liverpool database. For these unavailable drugs, the pharmacists (ES and DB) judged if there might be risk of a DDI. Pharmacokinetic parameters of these drugs were used (US FDA prescribing information and MicroMedex®) to evaluate these interactions. Overall, we defined Category 2 and 3 as the clinically relevant DDIs.[17]

Risk for drug-drug interactions per patient

To assess the number of patients at risk for a clinically relevant DDI, we counted the patients with at least one predicted DDI between co-medication and one of the DAA regimens. Further, we compared the risk for DDI between subgroups of patients: (a) patients aged < 65 years vs. ≥ 65 years[17], and (b) in patients with vs. without cirrhosis. We used Fib-4 index > 3.25 to classify patients as cirrhotic.[18]

Analyses

Descriptive analyses were performed with frequency counts and proportions. For the subgroup analyses we used chi-square tests. All analyses were performed in SPSS (IBM SPSS Statistics 20).

Results

Patients and use of co-medication

This cohort included 467 patients; we excluded 6 patients from the analysis because data on co-medication was missing. There were 313 males and the mean age was 51 years (Table 2). A total of 356 patients (77%) used co-medication at start of HCV therapy and 105 patients did not use any co-medication. The number of medications per patient ranged from 1 - 17 (median 2). Of the cohort, 12% used ≥6 medications at start of HCV therapy. Overall, the 356 patients had a total number of 1329 prescriptions (including CAMs), which comprised 260 different drugs (Figure 1). Most frequently used co-medication were antidepressants, proton pump inhibitors (PPIs), benzodiazepine derivatives, and drugs for opioid dependence (Table 3).

Table 2. Patient characteristics

Characteristic	Overall (n=461)
Age, years – mean (range)	51 (19-77)
Age ≥ 65 years – n (%)	30 (7)
Male sex – n (%)	313 (68)
Caucasian – n (%)[a]	316 (90)
Treatment naive – n (%)[b]	269 (58)
Decompensated liver disease – n (%)	23 (5)
FIB-4 index > 3.25 (cirrhosis) – n (%)[c]	115 (26)
Creatinine clearance < 30 ml/min – n (%)[d]	3 (1)

[a] Race: available in 352 patients; [b] previous response: available in 448 patients, [c] FIB-4 index: available in 437 patients, [d] creatinine clearance: available in 407 patients.

Table 3. Most frequently used (>2.0%) concomitant medications at start of hepatitis C treatment.

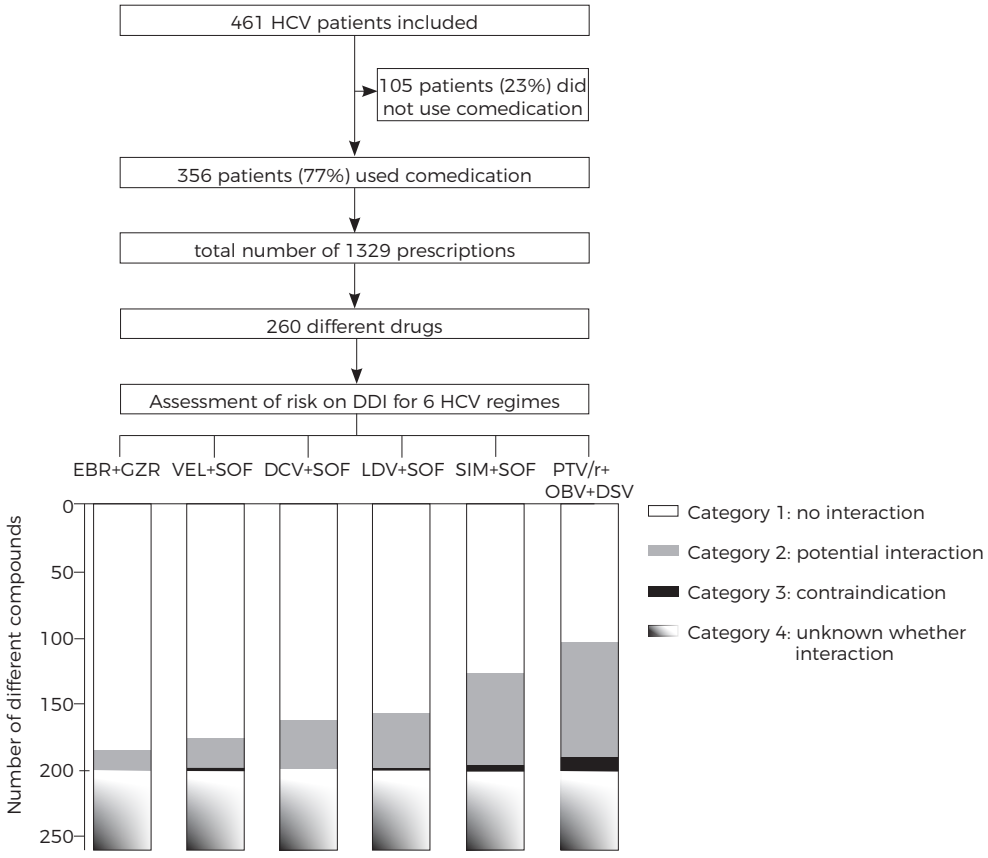
Drug class	ATC-code (4rd level)	n (%)[a]
Antidepressants (both tricyclic antidepressants and selective serontonin reuptake inhibitors (e.g. amitryptiline, sertraline)	N06AA, N06AB, N06AX	98 (7.4)
Proton pump inhibitors (e.g. omeprazole)	A02BC	94 (7.1)
Benzodiazepine derivatives (e.g. diazepam, flurazepam)	N05BA, N05CD	94 (7.1)
Drugs used in opioid dependence (e.g. methadone)	N07BC	74 (5.6)
Selective beta-2-adrenoreceptor agonists (respiratory agents both systemic and inhalants e.g. salbutamol)	R03AC, R03CC	55 (4.2)
Antipsychotics (e.g. olanzapine, risperidon)	N05AA, N05AB, N05AD, N05AF, N05AH, N05AL, N05AN, N05AX	46 (3.5)
Vitamin D and analogues (e.g. colecalciferol)	A11CC	38 (2.9)
Thiazides (e.g. hydrochlorothiazide)	C03AA	34 (2.6)
Selective beta blocking agents (e.g. metoprolol)	C07AB	32 (2.4)
ACE inhibitors (e.g. enalapril)	C09AA	32 (2.4)
Glucocorticoids (respiratory sytem e.g. beclometasone)	R03BA	32 (2.4)
Biguanides (e.g. metformin)	A10BA	27 (2.0)
Platelet aggregation inhibitors excl. heparin (e.g. acetylsalicylic acid)	B01AC	26 (2.0)
Dihydropyridine derivatives (calcium channel blockers e.g. amlodipne)	C08CA	26 (2.0)

[a] Percentage is calculated using the total number of prescriptions in this cohort (n = 1329), ACE: Angiotensin I converting enzyme, ATC: Anatomical Therapeutic Chemical

Predicted drug-drug interactions with direct-acting antivirals

We used our cohort to predict DDIs between co-medication and DAA regimens. Figure 1 presents the distribution of the DDI categories per DAA regimen for 260 different drugs. The combination of grazoprevir plus elbasvir and sofosbuvir plus velpatasvir had the lowest number of predicted DDIs in our mono-infected cohort. Grazoprevir plus elbasvir and sofosbuvir plus daclatasvir had no contraindicated drugs (Category 3) and no clinical significant interactions were predicted with 72% and 63%, respectively, of the concomitantly used drugs (Category 1).

Figure 1. Overview of concomitant medication and predicted number of drug-drug interactions (DDIs) between the direct-acting antiviral combinations of regimens and 260 different compounds



Sofosbuvir (SOF), simeprevir (SIM), and daclatasvir (DCV) are licensed as separate compounds for hepatitis C virus (HCV) infected patients. These drugs are separately available in the Liverpool database. However, we present these regimes together, because in clinical practice these drugs are used in combination. DDIs with PTV/r, OBV plus DSV (paritaprevir/ritonavir, ombitasvir plus dasabuvir), ledipasvir (LDV) plus SOF, velpatasvir (VEL) plus SOF, and elbasvir (EBR) plus grazoprevir (GZR) were available per combination in the Liverpool database.

The combination of paritaprevir/ritonavir, ombitasvir plus dasabuvir had the most contraindications (4%), followed by simeprevir (2%), and velpatasvir (1%). Category 2 interactions were also mainly predicted with the regimen containing paritaprevir/ritonavir, ombitasvir plus dasabuvir (33%) and sofosbuvir plus simeprevir (26%). Interestingly, ~90% of these category 2 DDIs have not been studied in vivo. These potential interactions were predicted by the pharmacologist of the University of Liverpool database, based on the characteristics of the drugs. The top 5 medications which can cause clinically relevant DDIs with at least one of the antiviral regimens are shown in Table 4.

The risk of DDIs could not be assessed in 60 of the 260 different drugs (Category 4), because the drugs were not listed in the University of Liverpool database (July 2016). The top three of therapeutic subgroup (2nd ATC level) in Category 4 were antihemorrhagics (B02; e.g. coagulation factors), vitamins (A11; e.g. colecalciferol), and psycholeptics (N05; e.g. flunitrazepam) which were used by a total of 17, 33, and 13 patients, respectively.

The pharmacists (ES and DB) judged if there were potential interactions with these 60 drugs and DAAs. We predicted that 11 drugs had a potential interaction, 30 drugs would not cause interactions, and for 19 drugs it is unknown if there is a potential interaction (for example: metabolism not known of the co-medication), data is shown in Table 5.

Table 4. Top five concomitant medication causing clinically relevant interactions with at least one of the antiviral regimens.

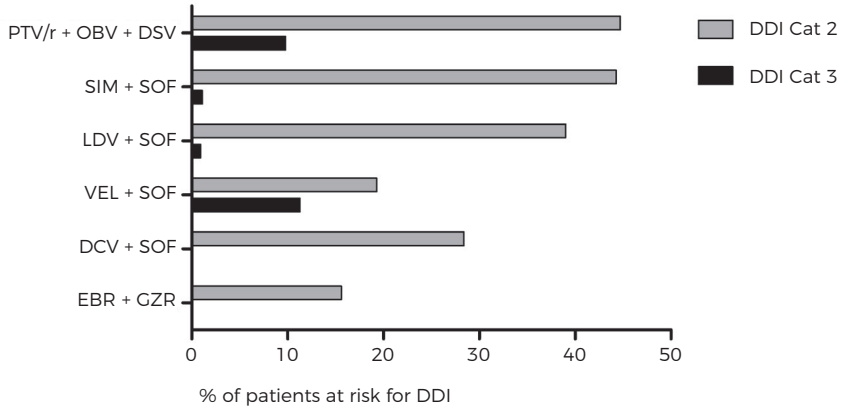
DDI category	Drug class	ATC code	n
DDI category 2	Benzodiazepines	N05BA	61
	Antidepressants	N06A	43
	Proton pump inhibitors (omeprazole)	A02BC	42
	Glucocorticoids respiratory system	R03BA	30
	Selective beta-blocking agents	C07AB	29
DDI category 3	Proton pump inhibitors (esomeprazole, pantoprazole)	A02BC	52
	HMG CoA reductase inhibitors (statins)	C10AA	19
	Antipsychotics	N05A	13
	Selective beta-2-adrenoreceptor agonists respiratory system	R03AC/ R03CC	12
	CAM	no ATC	2

CAM: complementary and alternative medicine, HMG CoA: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, DDI: drug-drug interaction, ATC: Anatomical Therapeutic Chemical

Risk for drug-drug interaction per patient

The majority of the patients in our cohort (60%) was at risk for a clinically relevant DDI with at least one of the DAA regimens: 93 patients (20%) used a drug that would be contraindicated (Category 3) and 184 patients (40%) had co-medication leading to a possible interaction (Category 2), which would require close monitoring, alternation of drug dosage or timing of administration. Figure 2 shows the risk of a DDI per DAA regimen per patient. The risk for DDIs per patient did not differ in patients aged below or above 65 years (60% vs. 67%, p=0.45), nor between patients without cirrhosis and with cirrhosis (60% vs. 64%, p= 0.50).

Figure 2. Risk on a clinically relevant drug-drug interaction per patient (n = 461), grouped per direct-acting antiviral regimen



Cat: category, DDI: drug-drug interaction, SIM = simeprevir, SOF = sofosbuvir, PTV/r = paritaprevir/ritonavir, OBV = ombitasvir, LDV = ledipasvir, DCV = daclatasvir, DSV = dasabuvir, EBR = elbasvir, GZR = grazoprevir, VEL = velpatasvir

Discussion

In this nationwide, real-life cohort study, we show that the majority of HCV-infected patients is at risk for having a clinically relevant DDI with new DAAs. This can have a negative influence on treatment outcomes and could potentially harm the patient.[1] In this cohort, patients with cirrhosis or ≥65 years old did not have a higher risk for a DDI when compared with patients <65 years old or without cirrhosis. This contrasts with a recently published study[17] and might be explained due to low number of elderly patients in our cohort and the lower mean age of patients ≥65 years (68 years, standard deviation (SD) 3). This shows that not only the elderly are at risk for a DDI. The psycho-active agents such as antidepressants (7.4%) and benzodiazepines (7.1%) were the most frequently used drugs in our cohort, as well as in the literature.[3] This is relevant because these drugs increase the risk for DDIs: antidepressants and benzodiazepines are extensively metabolized through CYP enzymes, which can be inhibited by DAAs.[21, 22] This causes increased plasma concentrations of psycho-active agents increasing the likelihood of toxicity.

PPIs were also responsible for many clinically relevant DDIs in our cohort, both as victim and perpetrator.[15] Omeprazole is a victim of paritaprevir/ritonavir, ombitasvir plus dasabuvir due to CYP2C19 induction of ritonavir, decreasing omeprazole exposure with 40-50%.[23] In contrast, PPIs are the perpetrators of a DDI with ledipasvir and velpatasvir. PPIs increase gastric pH, which decreases exposure to DAAs due to its insolubility at higher pH ranges.[24, 25] The clinical relevance for DDIs between PPIs and ledipasvir is under debate.[26, 27] For velpatasvir, the product label states that co- administration of omeprazole or other PPIs is not recommended, and that esomeprazole and pantoprazole are contraindicated.[25]

Table 5. Predicted drug-drug interactions and recommendations of drugs not available in the Liverpool database.

Generic name	ATC	Metabolism	Proposed interaction mechanism	Recommendation	Ref.
Barnidipine	C08CA12	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Barnidipine levels may increase, monitor for adverse reactions or monitor blood pressure.	19
Calcitriol	A11CC04	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Calcitriol levels may increase, monitor for adverse reactions.	*
Chlordiaze-poxide	N05BA02	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Chlordiazepoxide levels may increase, monitor adverse reactions.	*
Deferasirox	V03AC03	UGT1A1 and UGT1A3 substrate (CYP450-catalyzed metabolism: minor (8%)). MRP2, BCRP substrate	UGT1A1 inhibition by ombitasvir, paritaprevir/ritonavir. BCRP inhibition by ledipasvir, paritaprevir/ritonavir, velpatasvir.	Adverse reactions of deferasirox are dose dependent and frequently reported, therefore we advise to minimize the use of deferasirox during DAA therapy as increased plasma levels of deferasirox might occur.	20
		CYP3A4, CYP2C8, CYP1A2 inhibitor	Daclatasvir, dasabuvir, elbasvir, grazoprevir, paritaprevir/ritonavir, simeprevir, velpatasvir are substrate of CYP3A4. Dasabuvir is a substrate of CYP2C8.	Deferasirox inhibits CYP3A4 and CYP2C8 which might increase DAA levels, monitor adverse events.	
Phenpro-coumon	B01AA04	CYP2C9, CYP2C19 CYP1A2 CYP3A4 substrate	CYP2C19 inhibition by paritaprevir/ritonavir. CYP1A2 inhibiton by simeprevir. CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Inhibition of the various CYP enzymes may increase the phenprocoumon concentration, therefore monitor INR more frequently.	*
Isosorbide mononitrate	C01DA14	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Isosorbide mononitrate levels may increase, monitor for adverse reactions.	*
Levonorgestrel	G02BA03	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Levonorgestrel levels may increase, monitor for adverse reactions.	*

Generic name (continued)	ATC (continued)	Metabolism (continued)	Proposed interaction mechanism (continued)	Recommendation (continued)	Ref.
Medroxy-progesteron	G03AC06	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Medroxyprogesteron levels may increase, monitor for adverse reactions.	*
Misoprostol	A02BB01	Unknown	Pharmacodynamic interaction with ledipasvir. Misoprostol decreases gastrointestinal pH.	Based on studies with antacids: separate misoprostol and ledipasvir/sofosbuvir intake by 4 hours.	*
Oral contraceptives	G03A	Dependent on compounds. CYP3A4 metabolises e.g. levonorgestrel and cyproteron	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Dependent on the oral contraceptive agent there may be an interaction. Due to CYP inhibition of the DAAs the plasma levels of the contraceptive agent may increase with risk on toxicity. Switch to non-hormonal contraception during HCV therapy.	*
Solifenacin	G04BD08	CYP3A4 substrate	CYP3A4 inhibition by grazoprevir (?), paritaprevir/ritonavir, simeprevir.	Adverse reactions of solifenacin are dose dependent and solifenacin levels may increase when combined with CYP3A4 inhibitors. Adverse reactions are mostly anticholinergic. Preferably do not use during with CYP3A4 inhibitors.	*

* The product label and other sources such as Micromedex® Solutions and Lexicomp database (available via www.uptodate.com) were used to determine the pharmacokinetic profile and metabolism of the drugs. This information is used to predict theoretical interactions between the concomitant medication and DAAs (expert opinion ES and DB).
? Unknown whether the interaction is clinically relevant. HCV = hepatitis C virus, CYP = cytochrome P450, INR = international normalized ratio, DAA = direct-acting antiviral, ATC = anatomical therapeutic chemical, UGT = uridine diphosphate glucuronosyltransferase, MRP2 = multidrug resistance-associated protein 2, BCRP = breast cancer resistance protein, Ref = reference

Most frequently predicted DDIs were found with paritaprevir/ritonavir, ombitasvir plus dasabuvir, which fits with data from the published literature.[11] These interactions are predominantly caused by ritonavir, which strongly inhibits the most important drug-metabolizing enzyme (CYP3A4) and various other enzymes and drug-transporters are influenced (e.g. CYP2D6, P-glycoprotein (P-gp)).[28, 29] The fewest interactions were seen with the newest regimens: sofosbuvir plus velpatasvir and elbasvir plus grazoprevir. Elbasvir and grazoprevir are substrates of P-gp and CYP3A and only strong CYP3A inhibitors or inducers lead to clinically relevant DDIs. Grazoprevir is also a (weak) CYP3A inhibitor, but no DDIs between this combination and CYP3A substrates are listed.[30] However, we recommend caution when combining elbasvir and grazoprevir with CYP3A substrates with a narrow therapeutic range, such as tacrolimus.[31]

The contraindicated drugs count for up to 4% of the predicted interactions. This is a very clear signal to the physician: do not combine the co-medication with this DAA regimen. The dilemma is mostly present in the drugs categories in 2 and 4. In our study, ~90% of category 2 DDIs have not been studied, but were predicted by the University of Liverpool group. However, some DDIs cannot be predicted on theoretical grounds but do occur in clinical practice. For example, the unexpected severe bradycardia that occurred in nine patients who were on amiodarone treatment and received a sofosbuvir-containing regimen. The mechanism of this DDI and the role of sofosbuvir is still unclear.[6, 32-34] Further, 23% (n=60) of drugs used by patients from our cohort were not listed in the University of Liverpool database (category 4). We judged that 11 of these drugs might cause an enzymatic interaction with the currently used DAAs. Prescribers should be aware that when the drug is not mentioned in the database, it does not mean there is no interaction.

A strength of this study is that it is a nationwide multicenter cohort with a large number of patients. This cohort provides a representative overview of co-medication use in the Dutch HCV genotype 1 population with a treatment indication. Genotype 1 is globally the main genotype (60%) and we expect that the patients of the cohort reflect the patients who will be subjected to therapy.[35, 36] Further, we provide a risk assessment for drugs not available in the University of Liverpool database. Limitations of our study are the retrospective design and that our study describes predicted DDIs and not observed DDIs. Finally, the research question that led to this study was not the primary objective of data collection.

In conclusion, co-medication use is rich in both frequency and diversity in chronic HCV infected patients. DDIs may result in subtherapeutic or increased drug concentrations of DAAs or co-medication, and can cause treatment failure or toxicity. Physicians should be aware that the majority of patients are at risk for clinically relevant DDIs. In that case, co-medication can be adjusted prior to DAA therapy or DAA treatment can be aligned with co-medication use.

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Abbreviations

ACE	Angiotensin I Converting Enzyme
ATC	Anatomical Therapeutic Chemical
BCRP	Breast Cancer Resistance Protein
CAM	Complementary and Alternative Medicine
Cat	Category
CYP	CYtochrome P450
DAAs	Direct-acting Antivirals
DCV	Daclatasvir
DDIs	Drug-Drug Interactions
DSV	Dasabuvir
EBR	Elbasvir
FDA	U.S. Food and Drug Administration
GZR	Grazoprevir
HCV	Hepatitis C Virus
HMG CoA	3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A reductase
INR	International Normalized Ratio
LDV	Ledipasvir
MRP2	Multidrug Resistance-associated Protein 2
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
OATP	Organic Anion-Transporting Polypeptide
OBV	Ombitasvir
P-gp	P-glycoprotein
PPIs	Proton Pump Inhibitors
PTV/r	Paritaprevir/ritonavir
SIM	Simeprevir
SOF	Sofosbuvir
UGT	Uridine diphosphate GlucuronosylTransferase
VEL	Velpatasvir

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Identification of the best direct-acting antiviral regimen for patients with hepatitis C virus genotype 3 infection: a systematic review and network meta-analysis

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Abstract

Background & Aims

Direct-acting antivirals (DAA) are effective in treatment of chronic hepatitis C virus (HCV) infection, although results for patients infected with genotype 3 are suboptimal. There are several regimens available but direct comparisons have not been made and are unlikely to occur. We aimed to identify the most effective DAA regimen for patients infected with HCV genotype 3 and to assess the role of ribavirin.

Methods

We conducted a systematic search of Pubmed, Embase and Web of Science databases through March 2016. We performed a Bayesian network meta-analysis using a random effects model to indirectly compare regimens in patients with and without cirrhosis. We calculated mean estimated sustained virologic response (SVR) with 95% credible intervals (95% CrI) per regimen and effect of ribavirin as odds ratio. We focused on current recommended regimens and regimens under evaluation by regulatory authorities.

Results

Our search identified 2167 articles; 27 studies (comprising 3415 patients) were included. Among patients without cirrhosis, the greatest rates of SVR were estimated for those receiving sofosbuvir + velpatasvir with ribavirin (99%; 95% CrI, 98%–100%) and without ribavirin (97%; 95% CrI, 95%–99%), sofosbuvir + daclatasvir + ribavirin (96%; 95% CrI, 92%–98%), and sofosbuvir + peginterferon + ribavirin (95%; 95% CrI, 91%–98%), all for 12 weeks. Among patients with cirrhosis, the highest rates of SVR were estimated for those receiving sofosbuvir + velpatasvir for 24 weeks (96%; 95% CrI, 92%–99%), sofosbuvir + daclatasvir + ribavirin for 24 weeks (94%; 95% CrI, 87%–98%), and sofosbuvir + velpatasvir + ribavirin for 12 weeks (94%; 95% CrI, 86%–98%). Ribavirin increases efficacy in patients with and without cirrhosis (odds ratio, 2.6–4.5).

Conclusion

An indirect comparison of DAA-based treatments, using Bayesian network meta-analysis, found regimens containing sofosbuvir and velpatasvir to be the best option for patients with HCV genotype 3 infection. Our analyses indicate that ribavirin significantly increases rates of SVR and should be considered if tolerated.

Introduction

Chronic hepatitis C infection (HCV) represents a chronic liver condition that may lead to end-stage liver disease with cirrhosis and hepatocellular carcinoma.[1] The advent of direct-acting antiviral drugs (DAA) has completely changed the outlook of HCV. Viral cure has become possible with treatments that last 12-24 weeks and are devoid of side effects. The efficacy of DAA based therapy depends on patient related factors such as liver cirrhosis but also on viral genotype. Randomized clinical trials (RCTs) indicate that current regimens result in sustained virological response (SVR) in > 90% of patients with genotype 1.[2, 3] However, the evidence base for DAA therapies in genotype 3 is less extensive than for genotype 1. Also, treatment efficacy appears to be lower in genotype 3 patients, particularly in treatment experienced patients with cirrhosis.[4]

The expansion of DAAs prompted the FDA in 2013 to allow single-arm trials that lack formal placebo arms but instead use historical controls as a comparator.[5] This has resulted in an uneven trial landscape with multiple trials focusing on individual regimens. Key agents used in HCV genotype 3 patients are sofosbuvir, combined with ribavirin, daclatasvir, or velpatasvir. The comparative efficacy of individual combinations is largely unknown, mainly because of the paucity of head-to-head trials, while that information is necessary to steer guideline development and clinical decision making.

Our aim was to assess the comparative efficacy of all DAA regimens for HCV genotype 3 using a network meta-analysis and to determine whether addition of ribavirin to DAA improves treatment efficacy.

Methods

We conducted a systematic review and network meta-analysis according to an a priori written protocol. We used Preferred Reporting Items for Systematic Reviews and Network Meta-Analysis (PRISMA NMA) guidelines for this purpose.[6]

Systematic review

We performed a systematic search to identify studies in HCV genotype 3 patients treated with DAAs. Together with a medical librarian we designed the search strategy for the Pubmed, Embase and Web of Science databases, and conducted the final search on 15 March 2016 (Supplementary File 1). To include all available data we performed a manual search of abstract books of the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) conferences in 2015. Two researchers (FB and BA) independently screened articles following a dual pronged approach: screening on title and abstract and full text screening. Disagreements were resolved by a third researcher (WK). We included

studies (1) with patients above 18 years with HCV genotype 3, (2) RCTs, prospective clinical trials and/or real life studies with at least one DAA. We excluded studies (1) without results specified for HCV genotype 3, (2) where no sustained virological response (SVR) was reported, or (3) studies involving acute hepatitis C infected patients.

Outcomes, data extraction and risk of bias assessment

The primary outcome was the mean estimated probability of SVR per studied regimen. SVR was defined as an undetectable HCV RNA 12 weeks after cessation of treatment. One author (BA) extracted study characteristics and intention-to-treat SVR data per regimen and entered this in a structured electronic database (Castoredc ©). A second researcher cross checked all entered data (FB). Two authors (FB and BA) independently assessed the quality of studies using the Cochrane Collaboration risk of bias tool.[7] Disagreements were resolved by a third researcher (WK).

Statistical analysis

We conducted the network meta-analysis using Bayesian Markov Chain Monte Carlo (MCMC) methods. We used a random effects model with non-informative priors comparable to the network meta-analysis model of treatment response (NMA-TR) of Goring.[8] Direct and indirect evidence for all studied regimens were combined to estimate the probability of SVR per studied regimen, with a 95% equal tail Credible Interval (95%CrI), by means of a logistic regression model. We included the following fixed factors in the model: type of DAAs, ribavirin (binary), duration of therapy (12 or 16 or 24 weeks), presence of cirrhosis as prognostic factor for efficacy, and interaction of ribavirin and cirrhosis. As random effect we added the study, in order to model the positive correlation between study arms from the same study and to reflect deviations from the mean effects due to specific study and patient characteristics. The additional effect of ribavirin was estimated by means of an odds ratio (OR). The MCMC approach was based on 3 chains and updated with 200,000 simulations, thinning 1 per 10 and a burn-in of 10,000. We checked that the MCMC procedures had reached convergence by visually inspecting the history trace plots and the autocorrelation plots for irregularities.

To identify the most effective regimen, we focused on a subset of regimens recommended in guidelines, authorized in the market or under evaluation by regulatory authorities.[9-11] We ranked these regimens according to estimated SVR rates.[12] Further, we performed conventional meta-analyses per regimen to assess inconsistency and fit of the model. Heterogeneity was assessed by the estimated between-study variation τ^2 of the network meta-analysis and by I^2 of the meta-analyses per regimen.

Sensitivity analyses

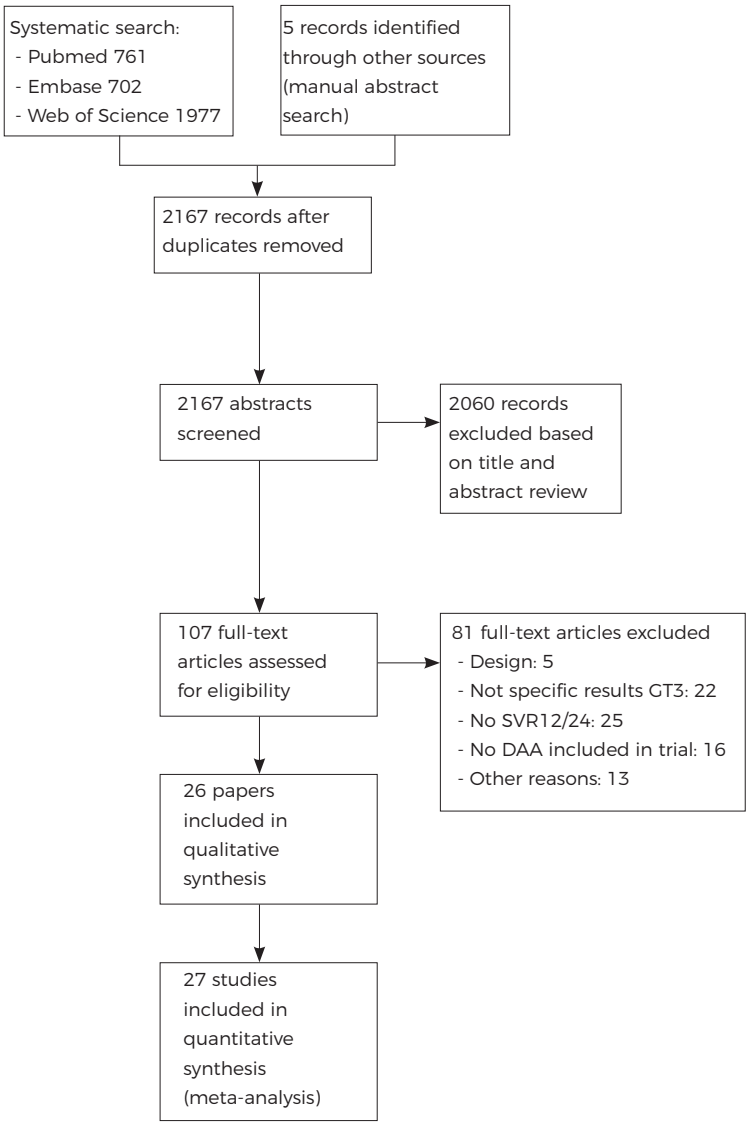
We performed four sensitivity analyses to assess the reliability and robustness of the model and to increase homogeneity of the population: (a) we included studies with a low risk of bias and studies with only a high risk of bias on blinding of participants as SVR is an objective outcome measure, (b) we included only regimens available in the market or under evaluation by regulatory authorities, (c) we excluded studies with patients with decompensated cirrhosis, and (d) we excluded studies with HIV/HCV co-infected patients. Because the effect of ribavirin is expected to be lower when added to a regimen consisting of 2 DAAs we did an additional analysis to assess the effect of ribavirin when combined with 2 DAAs. Treatment status (naive or experienced) was not included in the model because of limited available data specified on both cirrhosis status and treatment status per patient. To explore the effect of this choice, we performed an analysis with treatment status instead of cirrhosis in the model. All analyses were performed in WinBUGS 1.4.3 and R version 3.0.1 (R2winbugs).[13, 14] The WinBUGS syntax is available in Supplementary File 2.

Results

Treatment landscape

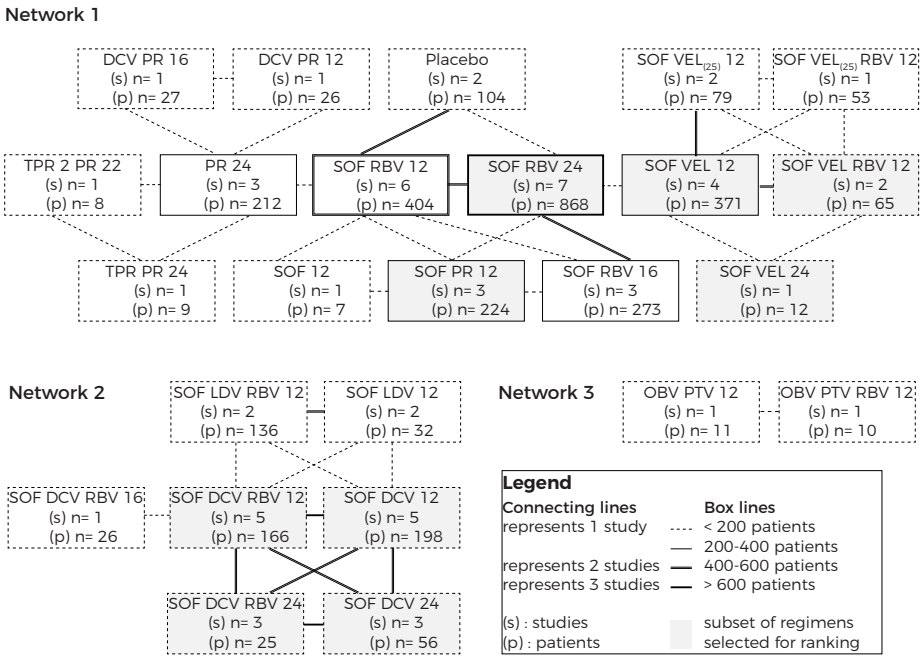
Of the 2167 identified articles, we selected 26 papers (21 full text, 5 abstracts) describing 27 studies (16 RCTs, 6 single arm studies, and 5 observational cohorts, Figure 1).[15-40] Overall, the 27 studies included 3415 patients, consisting of 2294 (67%) treatment naive patients and 1088 (32%) patients with cirrhosis. Eleven combinations of DAAs were studied, duration of therapy varied between 8 to 24 weeks with or without addition of ribavirin (Table 1 and Supplementary File 3). We excluded the 8 weeks regimens from our NMA, because only few patients were treated with 8 weeks regimens (n=13). There was variation in the number of patients studied per regimen, range 7-868). We designed three networks to connect regimens, but the majority of regimens were connected in network 1 and 2 (Figure 2).

Figure 1. Study selection flowchart



Study selection flowchart summarizing selection and identification of trials and studies.
GT3 = genotype 3, SVR12/24= sustained virological response at 12 or 24 weeks after cessation of treatment, DAA = direct acting antiviral.

Figure 2. Networks of studies



Evidence network of all DAA-based regimens studied in chronic hepatitis C genotype 3 patients. Thickness of the lines represent number of studies (connecting lines) or total number of patients studied (box lines). Within the box the DAA combination with duration (12, 16 or 24 weeks) is visible. Grey marked regimes are selected for ranking. Abbreviations: SOF = sofosbuvir, VEL = velpatasvir 100 mg, $VEL_{(25)}$ = velpatasvir 25 mg, RBV = ribavirin, PR = peginterferon and RBV, DCV = daclatasvir, LDV = ledipasvir, TPR = telaprevir, OBV = ombitasvir, PTV = paritaprevir/ritonavir, 12 = 12 weeks, 16 = 16 weeks, 24 = 24 weeks.

Sustained virological response in non-cirrhotic patients

Twenty-two different regimens were studied in non-cirrhotic HCV genotype 3 patients (Figure 3a). Highest SVR rates were estimated for sofosbuvir + daclatasvir + ribavirin for 24 weeks (98.9%, 95%CrI 97.6-99.6), sofosbuvir + velpatasvir + ribavirin for 12 weeks (98.8%, 95%CrI 97.5-99.6) and sofosbuvir + daclatasvir + ribavirin for 16 weeks (98.0%, 95%CrI 95.7-99.2).

We ranked a subset of the regimens (the clinically relevant regimens as based on guidelines and clinical practice, shown in Supplementary File 4 and marked grey in Figure 2) from 1 to 6. In this subset, the regimen sofosbuvir + velpatasvir + ribavirin for 12 weeks had highest probability to be ranked first, sofosbuvir + velpatasvir for 12 weeks to be ranked second, and sofosbuvir + daclatasvir + ribavirin for 12 weeks to be ranked third etcetera (Supplementary File 4a). Based on the ranking we estimated the differences in SVR rates between treatments (Table 2).

Table 1. Baseline characteristics of included studies

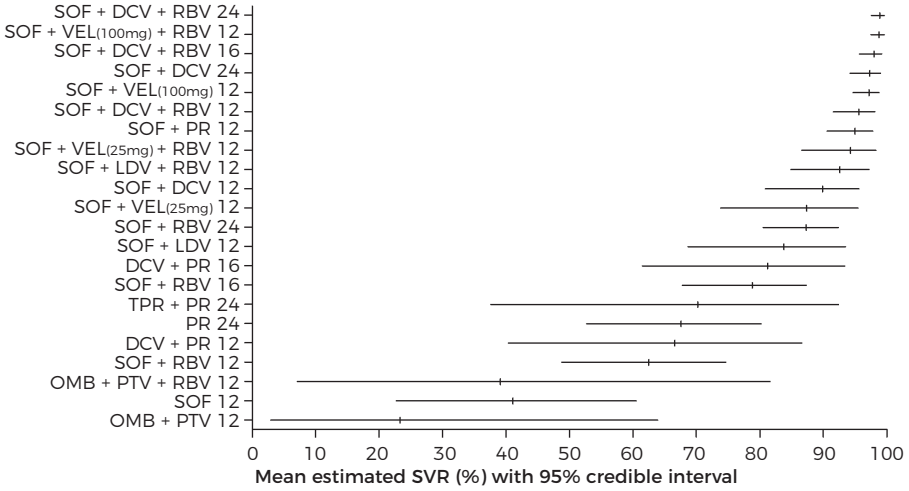
Author	Acronym / NCT number	Year	Location	Population and design	Intervention	Duration in weeks	N	Cirrhosis - n (%)	SVR - n (%)*
Foster [19]	NCT00561015	2011	EU	Phase IIa RCT, TN G2-3 pts	TPR + PR vs. PR	24	26	1 (4)	14(54)
Lawitz [26]	FISSION	2013	NA, EU, ANZ	Phase III RCT, TN G2-3 pts	SOF + RBV vs. PR	12-24	359	NR	212 (59)
Jacobson [25]	POSITRON	2013	NA, ANZ	Phase III RCT, TN G2-3 pts in which interferon was not an option	SOF + RBV vs. placebo	12	135	14 (10)	60 (61)
Jacobson [25]	FUSION	2013	NA, ANZ	Phase III RCT, TE G2-3 pts	SOF + RBV	12-16	127	49 (39)	58 (46)
Gane [23]	ELECTRON	2013	ANZ	Phase IIa RCT, TN G2-3 pts	SOF ± RBV / PR	12∫	42	0	40 (95)
Zeuzem [40]	VALENCE	2014	EU	Phase III RCT, later unblinded during study, TN and TE G2-3 pts	SOF + RBV vs. placebo	12-24	328	62 (19)	216 (83)
Sulkowski [37]	PHOTON-1	2014	NA	Phase III single arm study with TN and TE HCV/HIV coinfected G1-3 pts	SOF + RBV	12-24	59	12 (20)	44 (75)
Sulkowski [36]	AI444040	2014	NA	RCT, TN G1-3 pts	SOF + DCV ± RBV	24	18	0	16 (89)
Foster [18]	ASTRAL-3	2015	NA, EU, ANZ	Phase III RCT, TN and TE G3 pts	SOF + VEL vs. SOF + RBV	12	552	163 (30)	485 (88)
Foster [21]	BOSON	2015	NA, EU, ANZ	Phase III RCT, TN and TE G3 pts	SOF + RBV / PR	12-24	544	171 (31)	449 (83)
Pianko [33]	NCT01909804	2015	NA, ANZ	Phase II RCT, TE G1 and 3 pts	SOF + VEL(25mg and 100 mg) ± RBV	12	210	103 (49)	186 (89)
Nelson [32]	ALLY-3	2015	NA	Phase III single arm study, TN and TE G3 pts	SOF + DCV	12	152	32 (21)	135 (89)
Molina [31]	PHOTON-2	2015	EU, ANZ	Phase III single arm study, TN and TE HCV/HIV co-infected G1-4 pts	SOF + RBV	24	106	26 (25)	94 (89)
Gane [22]	NCT01826981	2015	ANZ	Phase II RCT (TN) and single arm (TE) study in pts with G3 or G6	SOF + LDV ± RBV	12	101	32 (32)	83 (82)
Dore [16]	AI444031	2015	NA, EU, ANZ	Phase IIb RCT, TN G2-3 pts	DCV + PR vs. PR	12-24	80	18 (23)	53 (66)

Everson [17]	NCT01858766	2015	NA	Phase II RCT, TN G1-6 pts	SOF + VEL (25mg and 100mg)	12	54	0	50 (93)
Curry [15]	ASTRAL-4	2015	NA	Phase III RCT, TN and TE G1-6 pts with decompensated cirrhosis	SOF + VEL ± RBV	12-24	39	39 (100)	24 (62)
Lawitz [27]	NR	2015	NA	Phase II single arm study, TE G2-3 pts	SOF + PR	12	24	12 (50)	20 (83)
Lawitz [28]	NCT01458535	2015	NA	Phase II trial with sequential enrollment, TN G1-3 pts	OMB + ABT-450/r ± RBV	12	21	0	6 (29)
Lin [30]	NR	2015	NR	Single arm study, G2-3 pts, decompensated cirrhosis was included	SOF + RBV	24	8	NR	6 (75)
Welzel [38]	CUP	2015	EU	Observational cohort (compassionate use program), G3 pts both HCV and HCV/HIV coinfected, decompensated cirrhosis was included	SOF + DCV ± RBV	12-24	24	22 (92)	22 (92)
Hezode [24]	ATU	2015	EU (France)	Observational cohort (compassionate use program), TN and TE G3 pts both HCV and HCV/HIV coinfected, decompensated cirrhosis was included	SOF + DCV ± RBV	12-24	78	NR†	70 (90)
Poordad [34]	ALLY-1	2015	NA	Single arm study, TN and TE G1-6 pts with advanced fibrosis or post-liver transplant HCV recurrence	SOF + DCV + RBV	12	17	17 (100)	15 (88)
Wyles [39]	ALLY-2	2015	NA	Phase II RCT (TN) and single arm (TE), G1-4 HCV/HIV coinfected pts	SOF + DCV	12 ∫	13	2 (15)	12 (92)
Foster [20]	EAP	2016	EU	Observational cohort (expanded access programme), G1-6 TN and TE, both HCV and HCV/HIV coinfected pts, decompensated cirrhosis was included	SOF + LDV ± RBV vs. SOF + DCV ± RBV	12	192	172 (90)	132 (69)
Shah [35]	NR	2016	Asia (India)	RCT, TN G1 and G3 pts	SOF + RBV	16-24	59	14 (24)	57 (97)
Leroy [29]	ALLY-3+	2016	EU, ANZ	Phase IIIb RCT, TN and TE G3 pts	SOF + DCV + RBV	12-16	50	36 (72)	45 (90)

NR = Not Reported, EU = Europe, NA= North America, ANZ = Australia and New-Zealand, TN = treatment naive, TE = treatment experienced, HCV= hepatitis C virus, HIV = human immunodeficiency virus, RCT = randomized clinical trial, pts = patients, G = genotype; Interventions: TPR = telaprevir, RBV = ribavirin, PR = peginterferon and RBV, SOF = sofosbuvir, DCV = daclatasvir, VEL = velpatasvir (100 mg unless otherwise indicated), LDV = ledipasvir, OMB = ombitasvir, ABT-450/r = paritaprevir/ritonavir; * in case of placebo, these patients are excluded from SVR calculations ; ∫ The treatment arms with 8 weeks duration are excluded from the analysis

Sofosbuvir + velpatasvir + ribavirin for 12 weeks reached higher SVR rates than all other recommended regimens in the subset (range 2-12% higher SVR). In contrast, sofosbuvir + velpatasvir for 12 weeks, sofosbuvir + daclatasvir + ribavirin for 12 weeks and sofosbuvir + peginterferon + ribavirin for 12 weeks had similar SVR rates. Sofosbuvir + ribavirin for 24 weeks had a similar estimated SVR to sofosbuvir + daclatasvir for 12 weeks; both were inferior to other reported regimens.

Figure 3a. Estimated SVR rates per regimen for non-cirrhotic patients



The figure shows the mean estimated probability on Sustained Virological Response (SVR) per regimen with 95%CrI. The SVR rates are estimated for patients without cirrhosis. Abbreviations: SOF = sofosbuvir, VEL = velpatasvir, RBV = ribavirin, PR = peginterferon and RBV, DCV = daclatasvir, LDV = ledipasvir, TPR = telaprevir, OBV = ombitasvir, PTV = paritaprevir/ritonavir, 12 = 12 weeks, 16 = 16 weeks, 24 = 24 weeks

Sustained virological response in cirrhotic patients

In total, 19 different regimens were studied in cirrhotic HCV genotype 3 patients. Highest SVR rates were estimated for: sofosbuvir + velpatasvir for 24 weeks (96.3%, 95%CrI 92.0-98.7), sofosbuvir + daclatasvir + ribavirin for 24 weeks (94.1%, 95%CrI 86.8-98.1) and sofosbuvir + velpatasvir + ribavirin for 12 weeks (93.7%, 95%CrI 85.9-98.0) (Figure 3b). Ranking and comparison of the subset of clinically important regimens resulted in sofosbuvir + velpatasvir for 24 weeks to be ranked first, sofosbuvir + daclatasvir + ribavirin for 24 weeks to be ranked second and sofosbuvir + velpatasvir + ribavirin for 12 weeks to be ranked third, etcetera (Supplementary File 4b). However when we compared the regimens in the subset to each other, similar SVR rates were estimated for the first three ranked regimens (Table 3). Sofosbuvir + velpatasvir for 12 weeks resulted in 7% lower SVR, while sofosbuvir + peginterferon + ribavirin for 12 weeks resulted in 16% lower SVR than sofosbuvir + velpatasvir + ribavirin for 12 weeks. Sofosbuvir + ribavirin for 24 weeks was inferior to all other regimens, with 22-40% lower SVR estimates.

Table 2. Mean difference in estimated SVR rate (%) between regimens in non-cirrhotic HCV genotype 3 patients (95%CrI)

Regimen SVR (95%CrI)	SOF+VEL+RBV 12 98.8 (97.5-99.6)	SOF+VEL 12 97.2 (94.7-98.8)	SOF+DCV+RBV 12 95.6 (91.6-98.1)	SOF+PR 12 95.0 (90.6-97.8)	SOF+DCV 12 89.9 (80.9-95.6)	SOF+RBV 24 87.3 (80.5-92.4)
1 **SOF+VEL+RBV 12** 98.8 (97.5-99.6)		**1.6 (0.4-3.5)***	**3.2 (0.7-7.1)***	**3.8 (1.1 – 8.1)***	**8.9 (3.0 – 18.2)***	**11.6 (6.8 – 18.0)***
2 **SOF+VEL 12** 97.2 (94.7-98.8)			1.5 (- 2.0 – 6.0)	2.2 (- 0.9 – 6.4)	**7.3 (1.5 – 16.3)***	**10.0 (5.5 – 16.0)***
3 **SOF+DCV+RBV 12** 95.6 (91.6-98.1)				0.6 (- 4.3 – 5.8)	**5.7 (1.3 – 12.7)***	**8.4 (2.0 – 15.7)***
4 **SOF+PR12** 95.0 (90.6-97.8)					5.1 (- 2.1 – 14.7)	**7.7 (3.3 – 13.2)***
5 **SOF+DCV 12** 89.9 (80.9-95.6)						2.7 (- 8.0 – 11.8)
6 **SOF+RBV 24** 87.3 (80.5-92.4)						

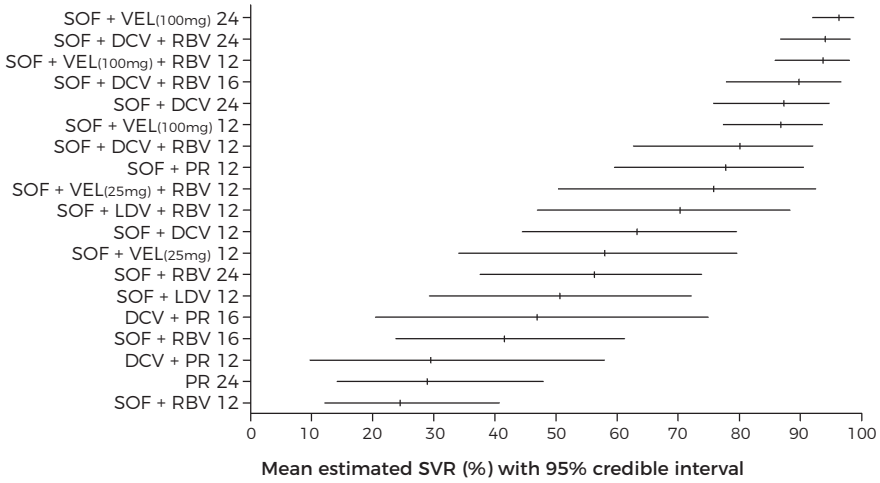
Subset of regimens are ordered based on ranking statistics (Supplementary File 4). Mean differences between 2 regimens are presented (with 95% Credible Interval). * and bold indicates a significantly higher estimated SVR rate. Abbreviations: SOF = sofosbuvir, VEL = velpatasvir 100 mg, RBV = ribavirin, PR = peginterferon and RBV, DCV = daclatasvir, 12 = 12 weeks, 24 = 24 weeks.

Table 3. Mean difference in estimated SVR rate (%) between regimens in cirrhotic HCV genotype 3 patients (95%CrI)

Regimen	SOF+VEL 24	SOF+DCV+ RBV 24	SOF+VEL+ RBV 12	SOF+DCV 24	SOF+VEL 12	SOF+DCV+ RBV 12	SOF+PR 12	SOF+RBV 24
SVR (95%CrI)	96.3 (92.0-98.7)	94.1 (86.8-98.1)	93.7 (85.9-98.0)	87.3 (75.8-94.7)	86.8 (77.4-93.6)	80.1 (62.7-92.0)	77.8 (59.6-90.5)	56.3 (37.6-73.8)
1 **SOF+VEL 24** 96.3 (92.0-98.7)		2.2 (- 2.5 – 8.9)	2.6 (- 0.9 – 8.5)	**9.0 (2.0 – 19.9)***	**9.5 (4.8 – 15.8)***	**16.2 (4.0 – 33.7)***	**18.5 (6.4 – 35.7)***	**40.0 (24.2 – 57.0)***
2 **SOF+DCV+ RBV 24** 94.1 (86.8-98.1)			0.4 (- 5.9 – 6.8)	**6.9 (1.9 – 14.4)***	7.3 (- 1.2 – 16.7)	**14.0 (5.7 – 25.7)***	**16.3 (4.0 – 33.1)***	**37.9 (22.2 – 54.3)***
3 **SOF+VEL+ RBV 12** 93.7 (85.9-98.0)				6.5 (- 3.8 – 18.9)	**7.0 (2.4 – 12.8)***	**13.6 (3.0 – 29.1)***	**15.9 (4.6 – 31.9)***	**37.4 (22.9 – 53.1)***
4 **SOF+DCV 24** 87.3 (75.8-94.7)					0.5 (- 12.7 – 11.5)	7.2 (- 2.8 – 20.5)	9.4 (- 6.2 – 27.8)	**31.0 (13.3 – 49.1)***
5 **SOF+VEL 12** 86.8 (77.4-93.6)						6.7 (- 7.2 – 24.3)	9.0 (- 3.4 – 25.4)	**30.5 (16.4 – 46.3)***
6 **SOF+DCV+ RBV 12** 80.1 (62.7-92.0)							2.3 (- 15.3 – 20.3)	**23.8 (5.8 – 42.1)***
7 SOF+PR 12 77.8 (59.6-90.5)								**21.6 (9.3 – 34.5)***
8 **SOF+RBV 24** 56.3 (37.6-73.8)								

Subset of regimens are ordered based on ranking statistics (Supplementary File 4). Mean differences between 2 regimens are presented (with 95% credible interval). * and bold indicates a significantly higher estimated SVR rate. Abbreviations: SOF = sofosbuvir, VEL = velpatasvir 100mg, RBV = ribavirin, PR = peginterferon and RBV, DCV = daclatasvir, 12 = 12 weeks, 24 = 24 weeks.

Figure 3b. Estimated SVR rates per regimen for cirrhotic patients



The figure shows the mean estimated probability on Sustained Virological Response (SVR) per regimen with 95% CrI. The SVR rates are estimated for patients with cirrhosis. Abbreviations: SOF = sofosbuvir, VEL = velpatasvir, RBV = ribavirin, PR = peginterferon and RBV, DCV = daclatasvir, LDV = ledipasvir, 12 = 12 weeks, 16 = 16 weeks, 24 = 24 weeks.

Effect of ribavirin

In patients without cirrhosis the OR of ribavirin was 2.6 (95%CrI 1.3-4.7), and in patients with cirrhosis 4.5 (95%CrI 2.5-7.7). We also performed an analysis with only studies including 2 DAAs +/- ribavirin. We found an OR of 6.5 (95%CrI 1.9-17.8) in patients without cirrhosis and OR 3.9 (95%CrI 2.0-7.0) in patients with cirrhosis. Ribavirin had a significant additional effect, even when used with 2 DAAs in both cirrhotic and non-cirrhotic patients.

Fit of the model and sensitivity analyses

To assess the consistency and fit of the model we performed direct meta-analyses of regimens and the results were largely similar to our network meta-analysis, with the exception of two regimens: sofosbuvir for 12 weeks and sofosbuvir + velpatasvir for 24 weeks. These 2 regimens were only studied once in 7 and 12 patients respectively.[15, 23] With regard to heterogeneity, the majority of meta-analyses per regimen had an I^2 <50%, except for 3 regimens (sofosbuvir + ribavirin 12 weeks, sofosbuvir + ribavirin 16 weeks (patients without cirrhosis), and sofosbuvir + velpatasvir(100mg) 12 weeks (patients with cirrhosis))(Supplementary File 5). The network meta-analysis resulted in an overall estimated τ^2 of 0.78 (95%CrI 0.27-1.73), suggesting between-study variation in the SVR rates. We performed four sensitivity analyses (Supplementary File 6). For the

first sensitivity analysis we excluded studies with a high risk of bias, except studies with only a high risk of bias on blinding of participants (Supplementary File 7). Overall estimated SVR rates were lower and 95%CrI wider; SVR for sofosbuvir + daclatasvir regimens dropped while sofosbuvir + velpatasvir regimens had similar SVR estimates as in the primary analysis. Results were consistent with the primary analysis in the other three sensitivity analyses. We also built a model where cirrhosis was replaced by treatment status (treatment naive vs. treatment experienced). Again, estimated SVR and ranking were largely similar to the overall results (Supplementary File 8).

Discussion

In this systematic review and network meta-analysis, we combined data from 27 studies to establish a hierarchy of available treatment regimens for HCV genotype 3. The key finding is that sofosbuvir-velpatasvir regimens achieve the highest efficacy in HCV genotype 3: sofosbuvir + velpatasvir + ribavirin for 12 weeks in non-cirrhotics and sofosbuvir + velpatasvir (without ribavirin) for 24 weeks or with ribavirin for 12 weeks in cirrhotics, although similar estimated SVR rates can be reached with sofosbuvir + daclatasvir + ribavirin for 24 weeks. In patients without cirrhosis, regimens such as sofosbuvir + peginterferon + ribavirin and sofosbuvir + daclatasvir + ribavirin for 12 weeks only had 1- 4% lower estimated SVR rates, and remain an option for treatment. The advantage of sofosbuvir + velpatasvir over other regimens is that ribavirin can be omitted in non-cirrhotics and that it shortens duration of treatment in cirrhotics.

Second, we established the added value of ribavirin (OR 2.6-4.5), regardless of the presence of cirrhosis. However, it is important to keep in mind that the actual effect of adding ribavirin on SVR rates depends on the efficacy of the backbone regimen: the increase in SVR due to ribavirin is highest with regimens that have a lower intrinsic efficacy. A recent review and current AASLD guidelines suggest that ribavirin can be dropped from the combination sofosbuvir + daclatasvir in non-cirrhotic genotype 3 patients. Our data suggest that in this case SVR drops by 6%. In clinical practice, the effect of ribavirin has to be traded off against both the side effect profile of ribavirin and the expected reduction in costs related to the DAA. The authors of this review and guideline recommend use of ribavirin in cirrhosis which is supported by our data.[9, 41]

A third finding is the identification of regimens that are clearly inferior in HCV genotype 3: sofosbuvir + daclatasvir for 12 weeks and sofosbuvir + ribavirin for 24 weeks in non-cirrhotics. Both achieved 5-12% lower SVR rates compared to other recommended regimens. In cirrhotics, differences in efficacy were more visible: sofosbuvir + ribavirin for 24 weeks was obviously inferior to other reported regimens (22-40% lower SVR rates) and should be considered obsolete.

The lack of head-to-head trials is an important issue for guideline developers and physicians, and drives researchers to perform network meta-analyses.[42] In the field of hepatitis C, some network meta-analyses have been performed to assess relative efficacy of DAAs in HCV genotype 1.[3, 43, 44] Because genotype is an important predictor for response, these results cannot be compared with genotype 3. The technique of network meta-analysis is not only of value in HCV, but has merits for other disease entities such as alcoholic hepatitis or Crohn's disease.[45-47] Results of network meta-analyses support physicians and guideline developers in decision making, but can also identify treatments which should be compared head-to-head, in our study this could be the first until fourth ranked regimes.

Our study comes with strengths and limitations. We combined all available evidence of DAA-regimens for HCV genotype 3 with use of Bayesian statistics. Current guidelines do not rank therapies as formal head to head trials are lacking. Our method enables identification of a hierarchy of therapies for HCV genotype 3. One of the limitations of our study is that results are based on extensive networks with in a few cases only one or no study per connection. This forced us to perform an arm-based rather than a comparison-based network meta-analysis. This approach is supported by the literature for HCV, but does impede inconsistency assessment.[8] However, our model produced similar outcomes as the conventional meta-analyses per regimen, which bolsters our conclusions. Further, estimated SVR rates of peginterferon and ribavirin therapy in our study do reflect SVR rates in the literature.[48] Another limitation of our network meta-analysis is the risk of conceptual heterogeneity, reflecting differences between trials which may impair comparability. We used several strategies to target heterogeneity: 1) we used a random effects model (by including a study effect in our model), 2) we have split the analyses for patients with and without cirrhosis, and 3) we performed sensitivity analyses to increase homogeneity, which showed similar results. Moreover SVR is an objective outcome which decreases the risk of heterogeneity.[49] Many studies in our network analysis have a high risk of bias. Exclusion of these studies in our sensitivity analysis resulted in a similar ranking of regimens, but estimated SVR rates were lower and 95%CrIs were wider. In real world, SVR rates might be compromised in view of the lower generalizability of trials, but we do not expect that the hierarchy is affected. Lastly, we were not able to assess publication bias formally, as the studies per regimen ranged from 1-7, nevertheless we do not expect publication bias as the field of HCV evolves rapidly and trial results are needed for evaluation by regulatory authorities.[7]

Implications for clinical practice

The findings of our network meta-analysis can be used to prioritize DAA regimens for HCV genotype 3 patients in guidelines and clinical practice. In patients without cirrhosis we focused on 12 week regimens and four regimens have an estimated SVR rate of 95% or higher: sofosbuvir + velpatasvir + ribavirin for 12 weeks is the best option, directly followed by sofosbuvir + velpatasvir, sofosbuvir + daclatasvir + ribavirin and

sofosbuvir + peginterferon + ribavirin for 12 weeks. Sofosbuvir + ribavirin for 24 weeks and sofosbuvir + daclatasvir for 12 weeks are inferior. In patients with cirrhosis sofosbuvir + velpatasvir with ribavirin for 12 weeks or without ribavirin for 24 weeks and sofosbuvir + daclatasvir + ribavirin for 24 weeks can be recommended. Sofosbuvir + velpatasvir for 12 weeks had 7-10% lower estimated SVR rates compared to other regimes so can be considered as alternative instead of recommended regimen in cirrhotic patients.[9] Sofosbuvir + ribavirin for 24 weeks is inferior to newer regimens. Our study also shows that ribavirin significantly increases the estimated SVR rates, however the precise effect is dependent on the actual DAA combination. In clinical practice, choice of treatment may depend on several factors, such as availability and price of DAAs, tolerance of ribavirin, risk of adverse events or drug-drug interactions, and presence of resistance associated substitutions.

Acknowledgements

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Abbreviations

95%CrI	95% credible interval
AASLD	American Association for the Study of Liver Diseases
DAA	Direct-Acting Antiviral
EASL	European Association for the Study of the Liver
HCV	Hepatitis C Virus
NMA-TR	Network Meta-Analysis model of Treatment Response
OR	Odds Ratio
RCT	Randomized Clinical Trial
SVR	Sustained Virological Response
Velpatasvir	Velpatasvir 100mg
Velpatasvir (25mg)	Velpatasvir 25 mg

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Supplementary Files

Supplementary File 1. Search strategy

We searched 3 databases (Pubmed, Embase, Web of Science) to identify all studies conducted in HCV genotype 3 patients. The search strategy included the following terms or synonyms: (chronic) hepatitis C, genotype 3, clinical trial (cochrane sensitivity-maximizing search), and pragmatic trial. The year 2004 was set as a limitation because there is no data about DAAs prior to 2004.

We performed the first search on 7 December 2015 and repeated the search on 15 March 2016. Search strategy with items found per date are shown below.

Pubmed

	Query	7-12-2015	15-3-2016
#4	Search ((((((((((randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized[tiab] OR placebo[tiab] OR drug therapy[sh] OR randomly[tiab] OR trial[tiab] OR groups[tiab] NOT (animals[mh] NOT humans[mh])))))))) OR ((pragmatic trial[tiab]) OR pragmatic trial)) AND (((((((HCV-2/3) OR ((((G3[tiab] OR GT3[tiab] OR Genotype 3*[tiab] OR genotypes 3*[tiab] OR Genotype 2/3*[tiab] OR Genotypes 2/3*[tiab])))) AND (("Hepatitis C, Chronic"[Mesh] OR Hepatitis c[tiab] OR HCV[tiab])))))))) Sort by: Relevance	741	20
#3	Search ((((((HCV-2/3) OR ((((G3[tiab] OR GT3[tiab] OR Genotype 3*[tiab] OR genotypes 3*[tiab] OR Genotype 2/3*[tiab] OR Genotypes 2/3*[tiab])))) AND (("Hepatitis C, Chronic"[Mesh] OR Hepatitis c[tiab] OR HCV[tiab])))))))) Sort by: Relevance	1357	71
#2	Search (pragmatic trial[tiab]) OR pragmatic trial Sort by: Relevance	2691	200
#1	Search (((((((randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized[tiab] OR placebo[tiab] OR drug therapy[sh] OR randomly[tiab] OR trial[tiab] OR groups[tiab] NOT (animals[mh] NOT humans[mh])))))) Sort by: Relevance	1715077	61868

Web of Science

Search	Items found 7-12-2015	Items found 15-3-2016	Query
# 5	1,705	272	#3 AND #2 AND #1 Refined by: PUBLICATION YEARS: (2015 OR 2006 OR 2014 OR 2009 OR 2012 OR 2008 OR 2013 OR 2005 OR 2011 OR 2004 OR 2010 OR 2007) Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Time-span=All years
# 4	2,262	2,262	#3 AND #2 AND #1 Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Time-span=All years
# 3	292,820	292,820	TOPIC: (((((((hepatitis c) OR (chronic hepatitis c) OR HCV OR (hepatitis C virus) OR (Hep* C) OR (hepatitis NEAR C))))))) Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Time-span=All years
# 2	2,996,678	2,996,678	TOPIC: (((((((clinical trial) OR (randomi?ed clinical trial) OR (randomi?ed controlled trial) OR (clinical study) OR (clinical research) OR (controlled clinical trial) OR placebo OR random* OR randomi?ed OR (clinical NEAR/3 trial) OR trial OR (pragmatic trial) OR (pragmatic clinical trial) OR (real world) OR (real life) OR (multicent* study) OR RCT)))))) Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Time-span=All years
# 1	187,811	187,811	TOPIC: (((genotypes NEAR/3 3) OR (genotype NEAR/3 3) OR G3 OR GT3 OR (GT NEAR/3 3) OR (G NEAR/3 3) OR (HCV NEAR/3 3))) Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Time-span=All years

Embase search (both searches combined) on 15-3-2016

#	Searches	Results
1	exp chronic hepatitis/	29091
2	(hepatitis adj C).ti,ab,kw.	87682
3	hep* c.ti,ab,kw.	88266
4	Hepatitis c virus.ti,ab,kw.	55326
5	hcv.ti,ab,kw.	73000
6	1 or 2 or 3 or 4 or 5	126732
7	genotypes 3.af.	292
8	"genotype 2/3".af.	495
9	genotype 3.af.	3078
10	"genotypes 2/3".af.	217
11	gt3.af.	456
12	g3.af.	20533
13	g?3.af.	41614
14	7 or 8 or 9 or 10 or 11 or 12 or 13	45286
15	"hcv-2/3".af.	52
16	"hcv 2/3".af.	52
17	"hcv2/3".af.	7
18	"hcv 3".af.	281
19	"hcv-3".af.	281
20	"hcv3".af.	8
21	exp hepatitis c virus genotype 3/	575
22	15 or 16 or 17 or 18 or 19 or 20 or 21	867
23	6 and 14	3431
24	22 or 23	3749
25	Clinical trial/	863634
26	Randomized controlled trial/	409625
27	Randomization/	70908
28	Single blind procedure/	22311
29	Double blind procedure/	131694
30	Crossover procedure/	47521
31	Crossover procedure/	47521
32	Randomi?ed controlled trial$.tw.	138191
33	Rct.tw.	20739

34	Placebo/	289574
35	Random allocation.tw.	1576
36	Randomly allocated.tw.	25282
37	Allocated randomly.tw.	2147
38	(allocated adj2 random).tw.	839
39	Single blind$.tw.	17802
40	Double blind$.tw.	169626
41	((treble or triple) adj blind$).tw.	589
42	Placebo$.tw.	240175
43	Prospective study/	338633
44	pragmatic.tw.	13321
45	"pragmatic clinical trial".tw.	92
46	"real world".tw,ti,ab,kw.	28781
47	"real life".tw,ti,ab,kw.	16689
48	25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47	1652489
49	Case study/	38811
50	Case report.tw.	317843
51	letter/	892418
52	49 or 50 or 51	1242044
53	48 not 52	1612479
54	24 and 53	702

Supplementary File 2. Model

This supplementary file shows the Winbugs code, the following components are shown:

- the full model
- the code for probability on SVR for regime 1 and 2 as an example
- OR for ribavirin in patients with and without cirrhosis
- Difference between 2 regimes (for example regime 1 with and without ribavirin in non-cirrhotics, for example regime 1 and 2 in cirrhotics)

Model for Winbugs

```
{
        for (s in 1 : 27){
        study_effects[s] ~ dnorm(0, study_tau)
        }
        for (i in 1 : 11){
                for (t in 1: N_study_per_regime[i]){
                        LP[i,t] <- a1 + a2*reg_dum2[i] + a3*reg_dum3[i] + a4*reg_
dum4[i] + a5*reg_dum5[i] + a6*reg_dum6[i] + a7*reg_dum7[i] + a8*reg_dum8[i] +
a9*reg_dum9[i] + a10*reg_dum10[i] + a11*reg_dum11[i] + b1*dur_16[i,t] + b2*dur_24[i,t] +
c*rbv[i,t] + d*cirr[i,t] + e*rbv[i,t]*cirr[i,t] + study_effects[studies[i,t]]
                        P[i,t] <- 1/ (1+exp(-1*LP[i,t]))
                        succes[i,t] ~ dbin(P[i,t], N_total[i,t])
                }
        }
a1 ~ dnorm(0, 0.01)
a2 ~ dnorm(0, 0.01)
a3 ~ dnorm(0, 0.01)
a4 ~ dnorm(0, 0.01)
a5 ~ dnorm(0, 0.01)
a6 ~ dnorm(0, 0.01)
a7 ~ dnorm(0, 0.01)
a8 ~ dnorm(0, 0.01)
a9 ~ dnorm(0, 0.01)
a10 ~ dnorm(0, 0.01)
a11 ~ dnorm(0, 0.01)
b1 ~ dnorm(0, 0.01)
b2 ~ dnorm(0, 0.01)
c ~ dnorm(0, 0.01)
d ~ dnorm(0, 0.01)
e ~ dnorm(0, 0.01)
study_sd ~ dunif(0.01, 10)
study_tau <- 1/(study_sd*study_sd)
P1_12_WR <- 1/(1+exp(-1*a1))
P2_12_WR <- 1/(1+exp(-1*(a1+a2)))
P1_16_WR <- 1/(1+exp(-1*(a1+b1)))
P2_16_WR <- 1/(1+exp(-1*(a1+a2+b1)))
P1_12_RBV <- 1/(1+exp(-1*(a1+c)))
P2_12_RBV <- 1/(1+exp(-1*(a1+a2+c)))
```

```
P1_16_RBV <- 1/(1+exp(-1*(a1+c+b1)))
P2_16_RBV <- 1/(1+exp(-1*(a1+a2+c+b1)))
P1_24_WR <- 1/(1+exp(-1*(a1+b2)))
P2_24_WR <- 1/(1+exp(-1*(a1+a2+b2)))
P1_24_RBV <- 1/(1+exp(-1*(a1+c+b2)))
P2_24_RBV <- 1/(1+exp(-1*(a1+a2+c+b2)))
P1_12_WRC <- 1/(1+exp(-1*(a1+d)))
P2_12_WRC <- 1/(1+exp(-1*(a1+a2+d)))
P1_16_WRC <- 1/(1+exp(-1*(a1+b1+d)))
P2_16_WRC <- 1/(1+exp(-1*(a1+a2+b1+d)))
P1_12_RBVC <- 1/(1+exp(-1*(a1+c+d)))
P2_12_RBVC <- 1/(1+exp(-1*(a1+a2+c+d)))
P1_16_RBVC <- 1/(1+exp(-1*(a1+c+b1+d)))
P2_16_RBVC <- 1/(1+exp(-1*(a1+a2+c+b1+d)))
P1_24_WRC <- 1/(1+exp(-1*(a1+b2+d)))
P2_24_WRC <- 1/(1+exp(-1*(a1+a2+b2+d)))
P1_24_RBVC <- 1/(1+exp(-1*(a1+c+b2+d)))
P2_24_RBVC <- 1/(1+exp(-1*(a1+a2+c+b2+d)))

OR_rbv <- exp(c)
OR_rbvcirr <- exp(c+e)

ZC1 <- P1_12_RBV – P1_12_WR
C1 <- P1_24_WRC – P2_24_RBVC

}
```

Supplementary File 3. Extensive baseline characteristics of included studies

Author	Acronym /NCT number	Year	Location	Population and design	Intervention	Duration in weeks	N	Cirrhosis - n (%)	Age, y – mean (range)	Male sex – n (%)	TN – n (%)	SVR - n (%)	No SVR due to no EOT response/ relapse/ missing∞
Foster	NCT00 561015	2011	EU	Phase IIa RCT, TN G2-3 pts	1. Placebo/PR 2 + PR 22 2. TPR 2 + PR 24 3. TPR/PR 2 + PR 22	24 26 24	9 8 9	1 (4) 0 0	39 (20-63)* 43 (31-60)* 44 (27-51)*	9 (100) 5 (63) 8 (89)	9 (100) 8 (100) 9 (100)	4 (44) 4 (50) 6 (67)	0/2/3 2/2/0 0/3/0
Lawitz	FISSION	2013	NA, EU, ANZ	Phase III RCT, TN G2-3 pts	1. PR (incl RBV 800 mg) 2. SOF + RBV (wb)	24 12	176 183	NR for G3	NR for G3	NR for G3	176(100) 183(100)	110 (63) 102 (56)	NR for G3
Jacobson	POSITRON	2013	NA, ANZ	Phase III RCT, TN G2-3 pts in which interferon was not an option	1. Placebo 2. SOF + RBV (wb)	12 12	37 98	0 14 (14)	NR for G3	NR for G3	37 (100) 98 (100)	0 60 (61)	NR for G3
Jacobson	FUSION	2013	NA, ANZ	Phase III RCT, TE G2-3 pts	1. SOF + RBV (wb) 2. SOF + RBV (wb)	12 16	64 63	26 (41) 23 (37)	NR for G3	NR for G3	0 0	19 (30) 39 (62)	NR for G3
Gane	ELEC-TRON	2013	ANZ	Phase IIa RCT, TN G2-3 pts	1. SOF + RBV (wb) 2. SOF/PR 4 + SOF + RBV (wb) 8 3. SOF/PR 8 + SOF + RBV (wb) 4 4. SOF + PR (RBV wb) 5. SOF 6. SOF + PR (RBV wb)	12 12 12 12 12 8	6 6 6 7 7 10	0 0 0 0 0 0	NR for G3	NR for G3	6 (100) 6 (100) 6 (100) 7 (100) 7 (100) 10 (100)	6 (100) 6 (100) 6 (100) 6 (100) 5 (71) 10 (100)∫	0/0/0 0/0/0 0/0/0 0/0/0 0/2/0 0/0/0
Zeuzem	VALENCE	2014	EU	Phase III RCT, later unblinded during study, TN and TE G2-3 pts	1. Placebo 2. SOF + RBV (wb) 3. SOF + RBV (wb)	12 12 24	67 11 250	0 0 60 (24)	NR for G3 46 (30-59) 48 (19-69)	NR for G3 6 (55) 155 (62)	NR for G3 2 (18) 105 (42)	0 3 (27) 213 (85)	n/a 0/6/2 1/34/2
Sulkow-ski	PHOTON-1	2014	NA	Phase III single arm study with TN and TE HCV/HIV co-infected G1-3 pts	1. SOF + RBV (wb) 2. SOF + RBV (wb)	12 24	42 17	6 (14) 6 (35)	<50y: 23 < 50y: 3	34 (81) 14 (82)	42 (100) 0	28 (67) 16 (94)	0/12/2 0/1/0

Sulkow-ski	AI444040	2014	NA	RCT, TN G1-3 pts	1.	SOF 1 + SOF/DCV 23	24	7	0	NR for G3	NR for G3	7 (100)	5 (71)	1/1/0
					2.	SOF + DCV	24	6	0			6 (100)	6 (100)	0/0/0
					3.	SOF + DCV + RBV(800mg)	24	5	0			5 (100)	5 (100)	0/0/0
Foster	ASTRAL-3	2015	NA, EU, ANZ	Phase III RCT, TN and TE G3 pts	1.	SOF + RBV (wb)	24	275	83 (30)	50 (19-74)	174 (63)	204 (74)	221 (80)	10/38/6
					2.	SOF + VEL	12	277	80 (28)	49 (21-76)	170 (61)	206 (74)	264 (95)	0/11/2
Foster	BOSON	2015	NA, EU, ANZ	Phase III RCT, TN and TE G2-3 pts	1.	SOF + PR (RBV wb)	12	181	58 (32)	<65y: 172	121 (67)	94 (52)	168 (93)	0/9/4
					2.	SOF + RBV (wb)	16	181	57 (31)	<65y: 176	124 (69)	91 (50)	128 (71)	0/50/3
					3.	SOF + RBV (wb)	24	182	56 (31)	<65y: 179	118 (65)	94 (52)	153 (84)	3/24/2
Pianko	NCT01 909804	2015	NA, ANZ	Phase II RCT, TE G1 and 3 pts	Non-cirrhotic pts									
					1.	SOF + VEL (25mg)	12	26	0	54 (22-69)	18 (69)	0	22 (85)	0/4/0
					2.	SOF + VEL (25mg) + RBV (wb)	12	28	0	51 (25-67)	22 (79)	0	27 (96)	0/1/0
					3.	SOF + VEL (100mg)	12	27	0	55 (32-68)	18 (67)	0	27 (100)	0/0/0
					4.	SOF + VEL (100mg) + RBV (wb)	12	26	0	56 (42-72)	17 (65)	0	26 (100)	0/0/0
					Cirrhotic pts									
					5.	SOF + VEL (25mg)	12	26	26 (100)	57 (40-68)	21 (81)	0	15 (58)	0/11/0
					6.	SOF + VEL (25mg) + RBV (wb)	12	25	25 (100)	56 (38-65)	15 (60)	0	21 (84)	1/3/0
					7.	SOF + VEL (100mg)	12	26	26 (100)	56 (45-68)	20 (77)	0	23 (88)	0/3/0
					8.	SOF + VEL (100mg) + RBV (wb)	12	26	26 (100)	54 (44-65)	20 (77)	0	25 (96)	0/1/0
Nelson	ALLY-3	2015	NA	Phase III single arm study, TN and TE G3 pts	1.	SOF + DCV	12	152	32 (21)	55 (24-73)*	90 (59)	101 (66)	135 (89)	1/16/0
Molina	PHOTON-2	2015	EU, ANZ	Phase III single arm study, TN and TE HCV/HIV co-infect-ed G1-4 pts	1.	SOF + RBV (wb)	24	106	26 (25)	<50y: 67	76 (72)	57 (54)	94 (89)	1/10/1
Gane	NCT01 826981	2015	ANZ	Phase II RCT (TN) and single arm (TE) study in pts with G3 or G6	1.	SOF + LDV	12	25	4 (16)	43 (SD 10.2)	13 (52)	25 (100)	16 (64)	1/8/0
					2.	SOF + LDV + RBV (wb)	12	26	6 (23)	48 (SD 9.2)	11 (42)	26 (100)	26 (100)	0/0/0
					3.	SOF + LDV + RBV (wb)	12	50	22 (44)	52 (SD 8.2)	39 (78)	0	41 (82)	1/8/0
Dore	AI444031	2015	NA, EU, ANZ	Phase IIb RCT, TN G2-3 pts	1.	DCV + PR (RBV 800 mg)	12	26	7 (27)	46 (28-61)*	19 (73)	26 (100)	18 (69)	0/7/1
					2.	DCV + PR (RBV 800 mg)	16	27	4 (15)	44 (31-67)*	22 (82)	27 (100)	21 (78)	0/7/1
					3.	PR + placebo (RBV 800 mg)	24	27	7 (26)	46 (20-62)*	16 (59)	27 (100)	14 (52)	1/3/6

Author (cont.)	Acronym /NCT number (cont.)	Year (cont)	Location (cont.)	Population and design (cont.)	Intervention (cont.)	Duration in weeks (cont.)	N (cont)	Cirrhosis - n (%) (cont.)	Age, y – mean (range) (cont.)	Male sex – n (%) (cont.)	TN – n (%) (cont.)	SVR - n (%) (cont.)	No SVR due to no EOT response/ relapse/ missing∞ (cont.)
Everson	NCT01 858766	2015	NA	Phase II RCT, TN G1-6 pts	1. SOF + VEL (25mg) 2. SOF + VEL (100 mg)	12 12	27 27	0 0	52 (25-70) 50 (20-70)	18 (67) 17 (63)	27 (100) 27 (100)	25 (93) 25 (93)	1/1/0 0/2/0
Curry	ASTRAL-4	2015	NA	Phase III RCT, TN and TE G1-6 pts with decompensated cirrhosis	1. SOF + VEL 2. SOF + VEL 3. SOF + VEL + RBV (wb)	12 24 12	14 12 13	14 (100) 12 (100) 13 (100)	NR for G3	NR for G3	NR for G3	7 (50) 6 (50) 11 (85)	0/6/1 1/4/1 1/1/0
Lawitz	NR	2015	NA	Phase II single arm study, TE G2-3 pts	1. SOF + PR (RBV wb)	12	24	12 (50)	54 (39-64)	18 (75)	24 (100)	20 (83)	0/2/2
Lawitz	NCT01 458535	2015	NA	Phase II trial with sequential enrollment, TN G1-3 pts	1. OMB + ABT-450 2. OMB + ABT-450 + RBV (wb)	12 12	11 10	0 0	49 (SD 8.9) 40 (SD 13.1)	7 (64) 7 (70)	11 (100) 10 (100)	1 (10) 5 (50)	9/1/0 3/2/1
Lin	NR	2015	NR	Single arm study, G2-3 pts, decompensated cirrhosis was included	1. SOF + RBV (dose NR)	24	8	NR †	NR	NR	NR	6 (75)	?/?/?
Welzel	CUP	2015	EU	Observational cohort (compassionate use program), G3 pts both HCV and HCV/HIV co-infected, decompensated cirrhosis was included	1. SOF + DCV 2. SOF + DCV 3. SOF + DCV + RBV (dose NR) 4. SOF + DCV + RBV (dose NR)	12 24 12 24	3 8 3 7	3 (100) 8 (100) 3 (100) 7 (100)	NR	NR	NR	3 (100) 8 (100) 3 (100) 6 (86)	0/0/0 0/0/0 0/0/0 ?/1/?
Hezode	ATU	2015	EU (France)	Observational cohort (compassionate use program), TN and TE G3 pts both HCV and HCV/HIV coinfected, decompensated cirrhosis was included	1. SOF + DCV 2. SOF + DCV 3. SOF + DCV + RBV (dose NR) 4. SOF + DCV + RBV (dose NR)	12 24 12 24	26 35 4 13	22 (85) 35 (100) 3 (75) 13 (100)	NR	NR	NR	22 (85) 32 (91) 4 (100) 12 (92)	No VB, 6 relapse, 6 unknown

Poordad	ALLY-1	2015	NA	Single arm study, TN and TE G1-6 pts with advanced fibrosis or post-liver transplant HCV recurrence	1.	Advanced cirrhosis cohort : SOF + DCV + RBV (600 mg)	12	6	6 (100)	NR	NR	NR	5 (83)	?/?/?
					2.	Posttransplant cohort: SOF + DCV + RBV (600 mg)	12	11	NR †				10 (91)	?/?/?
Wyles	ALLY-2	2015	NA	Phase II RCT (TN) and single arm (TE), G1-4 HCV/HIV coinfected pts	1.	SOF + DCV	8	3	1 (33)	NR for G3	NR for G3	3 (100)	2 (67) ∫	0/1/0
					2.	SOF + DCV	12	6	0			6 (100)	6 (100)	0/0/0
					3.	SOF + DCV	12	4	1 (25)			0	4 (100)	0/0/0
Foster	EAP	2016	EU	Observational cohort (expanded access programme), G1-6 TN and TE, both HCV and HCV/HIV coinfected patients, decompensated cirrhosis was included	1.	SOF + DCV	12	7	5 (71)	(arm1+2):52*	(arm1+2): 90 (72)	(arm1+2): 49 (39)	5 (71)	(arm1+2): 0/18/16
					2.	SOF + DCV + RBV (dose NR)	12	118	115 (97)				86 (73)	
					3.	SOF + LDV	12	7	7 (100)	(arm3+4):54*	(arm3+4): 44 (66)	(arm3+4): 30 (45)	2 (29)	(arm3+4): 1/20/5
					4.	SOF + LDV + RBV (dose NR)	12	60	59 (98) ‡				39 (65)	
Shah	NR	2016	Asia (India)	RCT, TN G1 and G3 pts	1.	SOF + RBV (wb)	16	29	7 (24)	NR	NR	29 (100)	29 (100)	0/0/0
					2.	SOF + RBV (wb)	24	30	7 (23)			30 (100)	28 (93)	0/1/1
Leroy	ALLY-3+	2016	EU, ANZ	Phase IIIb RCT, TN and TE G3 pts	1.	SOF + DCV + RBV (wb)	12	24	18 (75)	53 (36-73)*	18 (75)	6 (25)	21 (88)	0/2/1
					2.	SOF + DCV + RBV (wb)	16	26	18 (69)	56 (42-62)*	22 (85)	7 (27)	24 (92)	0/2/0

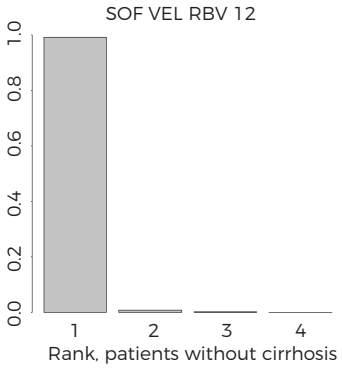
NR = Not Reported, EU = Europe, NA= North America, ANZ = Australia and New-Zealand, TN = treatment naive, TE = treatment experienced, HCV= hepatitis C virus, HIV = human immunodeficiency virus, RCT = randomized clinical trial, NR = not reported, G=genotype, pts = patients, y = years.
Interventions: TEL = telaprevir, IFN = peginterferon, RBV = ribavirin (dose: xxx mg or wb = weight based dose of RBV: 1000 mg daily if body weight < 75 kg, and 1200 mg daily if body weight ≥ 75 kg), SOF = sofosbuvir, DCV = daclatasvir, VEL = velpatasvir, LDV = ledipasvir, OMB = ombitasvir, ABT-450 = paritaprevir, PR = Peginterferon and RBV.
†All patients counted as cirrhosis.
‡ Patients with decompensated liver disease and orthoptic liver transplant were counted as cirrhosis, while patients with extrahepatic indication were counted as non-cirrhosis
∫ The treatment arms with 8 weeks duration are not included in the analysis
* median (range)
∞ Reasons for no end of treatment (EOT) response include: viral breakthrough (VB), non-response, early discontinuation; Reasons for missing include: lost to follow-up, withdrew consent, died

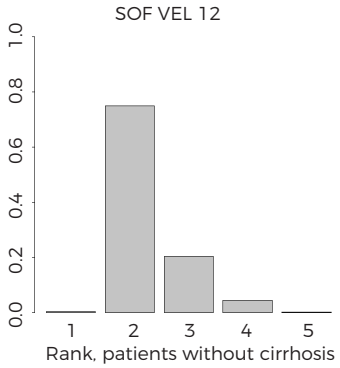
Supplementary File 4

a) Patients without cirrhosis

- Subset of regimens recommended in guidelines (EASL, AALSD, WHO) or under evaluation of regulatory authorities:
  Italic regimens are recommended in latest update of AASLD guideline (6 July 2016)
  1. *SOF + DCV 12*
  2. SOF + PR 12
  3. SOF + RBV 24
  4. *SOF + DCV + RBV 12*
  5. *SOF + VEL 12*
  6. *SOF + VEL + RBV 12*

- Rankograms for subset of regimens patients without cirrhosis:

1.


2.


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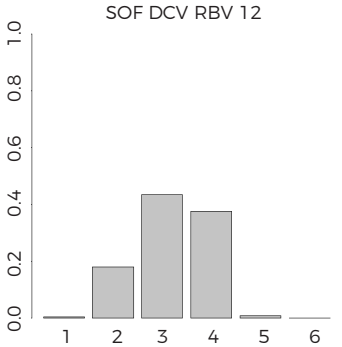

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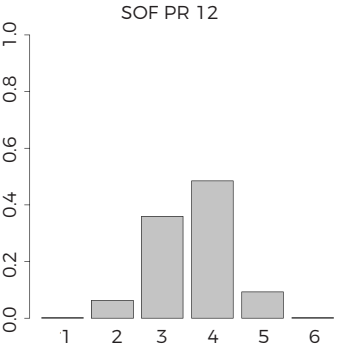

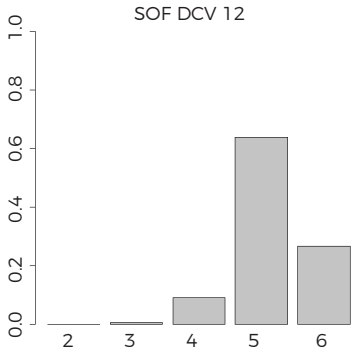

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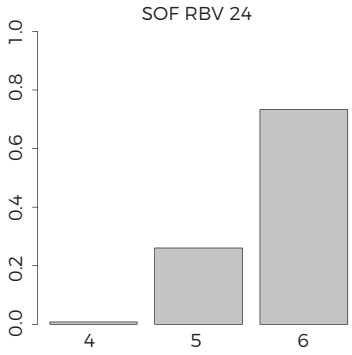
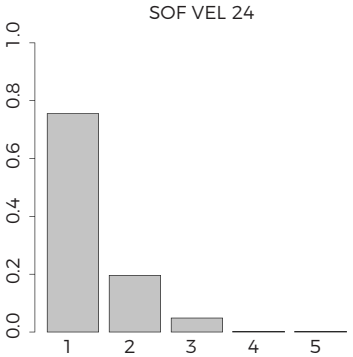


6.

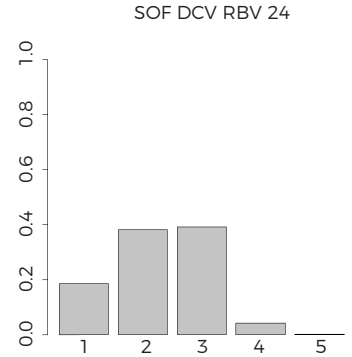
b) Patients with cirrhosis

- Subset of regimens recommended in guidelines (EASL, AALSD, WHO) or under evaluation of regulatory authorities:
  Italic regimens are recommended in latest update of AASLD guideline (6 July 2016)
  1. *SOF + DCV + RBV 24*
  2. *SOF + DCV 24*
  3. SOF + PR 12
  4. SOF + RBV 24
  5. SOF + DCV + RBV 12
  6. SOF + VEL 24
  7. *SOF + VEL + RBV 12*
  8. *SOF + VEL 12*

- Rankograms for subset of regimens in cirrhotic patients:



1.



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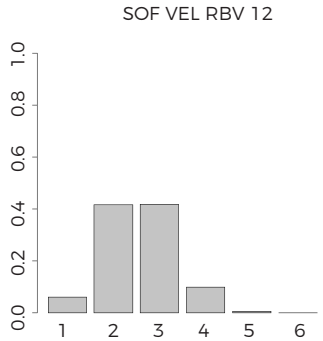
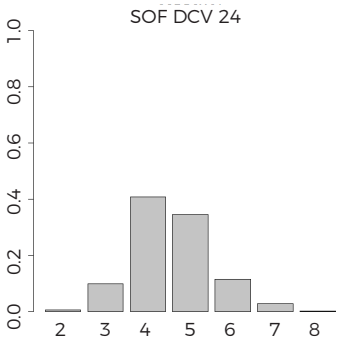
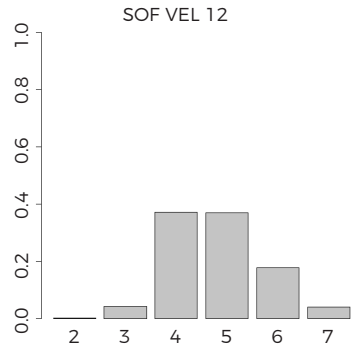

SOF VEL RBV 12
0.0
0.2
0.4
0.6
0.8
1.0
1 2 3 4 5 6
Rank, patients with cirrhosis

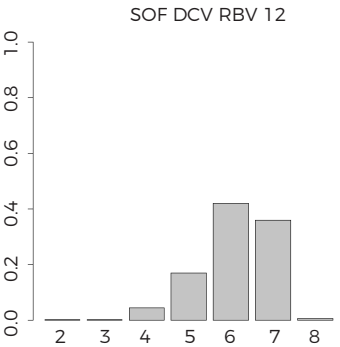
3.


SOF DCV 24
0.0
0.2
0.4
0.6
0.8
1.0
2 3 4 5 6 7 8
Rank, patients with cirrhosis

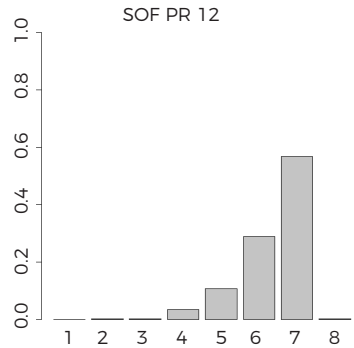
4.


SOF VEL 12
0.0
0.2
0.4
0.6
0.8
1.0
2 3 4 5 6 7
Rank, patients with cirrhosis

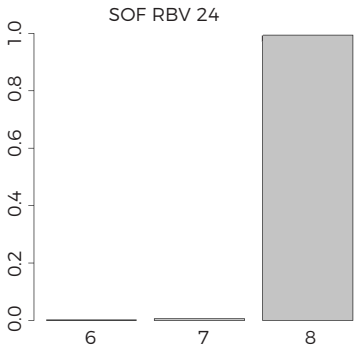
5.


SOF DCV RBV 12
0.0
0.2
0.4
0.6
0.8
1.0
2 3 4 5 6 7 8
Rank, patients with cirrhosis

6.


SOF PR 12
0.0
0.2
0.4
0.6
0.8
1.0
1 2 3 4 5 6 7 8
Rank, patients with cirrhosis

7.

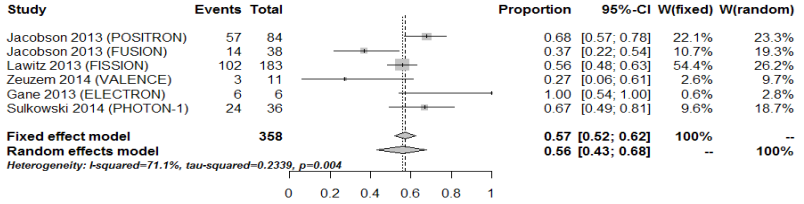

SOF RBV 24
0.0
0.2
0.4
0.6
0.8
1.0
6 7 8
Rank, patients with cirrhosis

8.

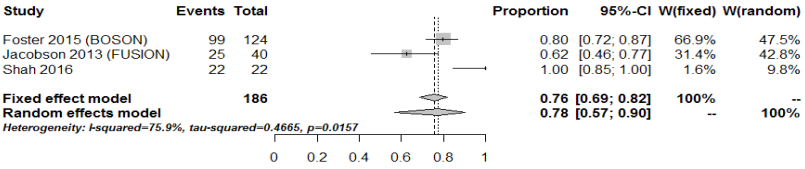
Supplementary File 5.

Meta-analyses per studied regimen in patients without cirrhosis (only regimens with >1 study are shown).

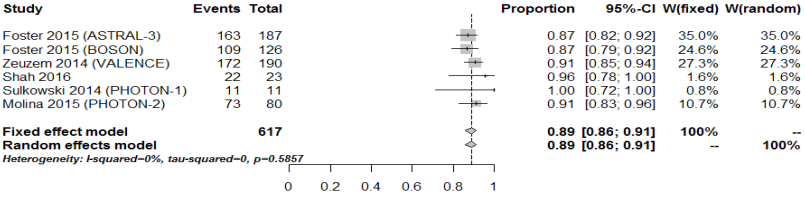
SOF RBV 12



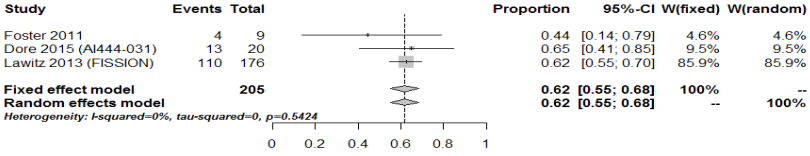
SOF RBV 16



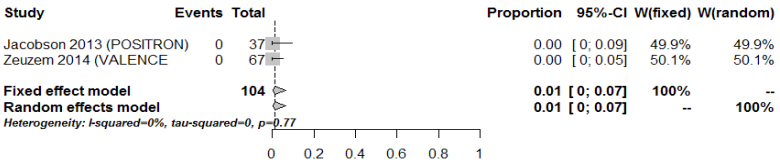
SOF RBV 24



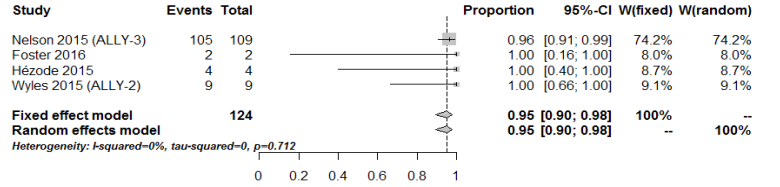
PR 24



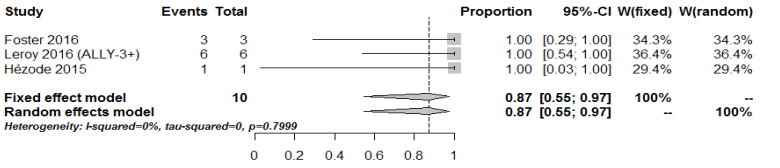
Placebo



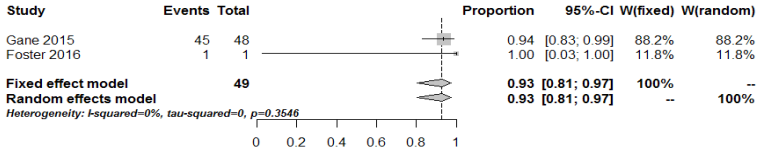
SOF DCV 12



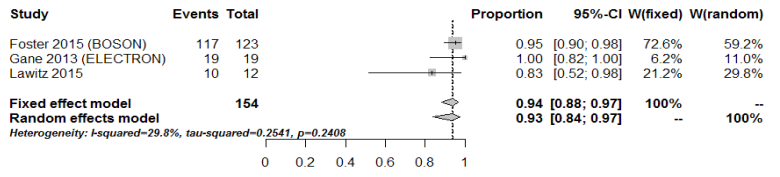
SOF DCV RBV 12



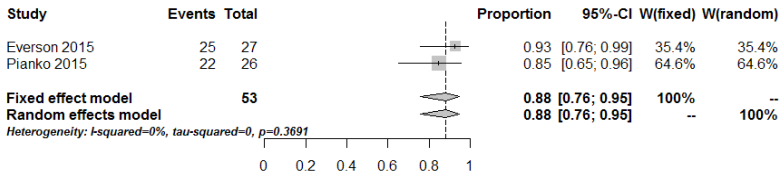
SOF LDV RBV 12



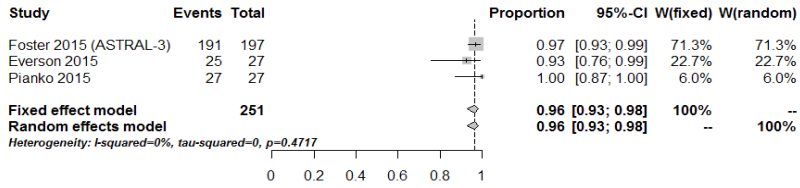
SOF PR 12



SOF VEL(25mg) 12

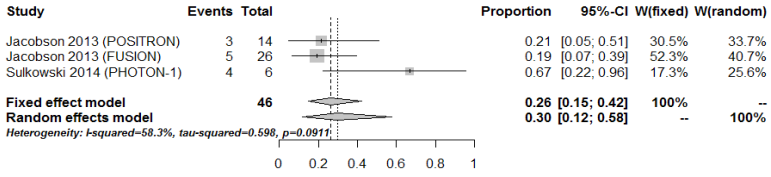


SOF VEL(100mg) 12

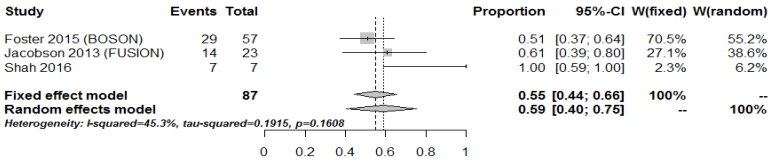


Meta-analyses per studied regimen in patients with cirrhosis (only regimens with >1 study are shown).

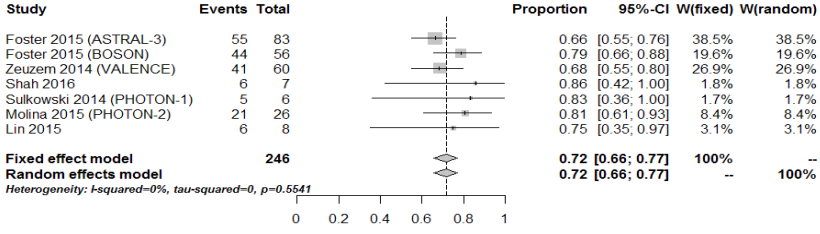
SOF RBV 12



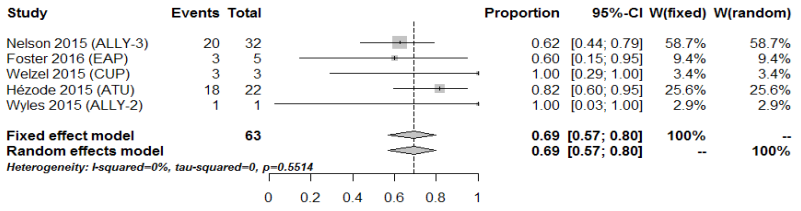
SOF RBV 16



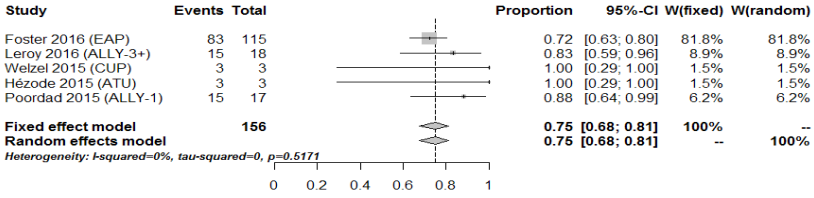
SOF RBV 24



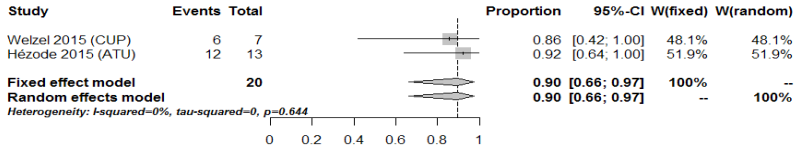
SOF DCV 12



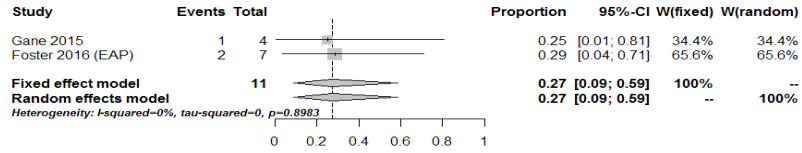
SOF DCV RBV 12



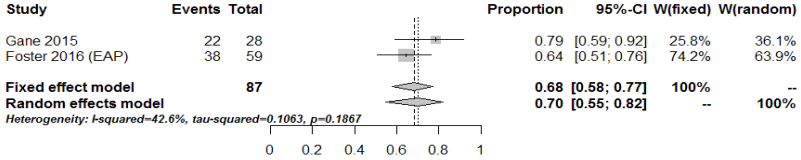
SOF DCV RBV 24



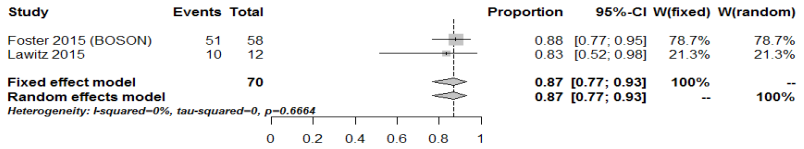
SOF LDV 12



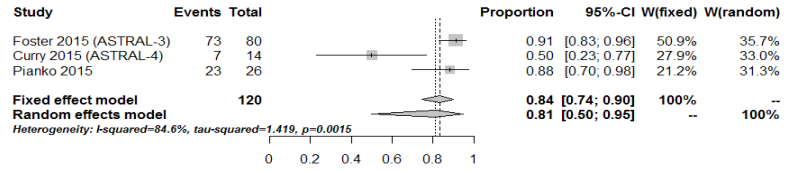
SOF LDV RBV 12



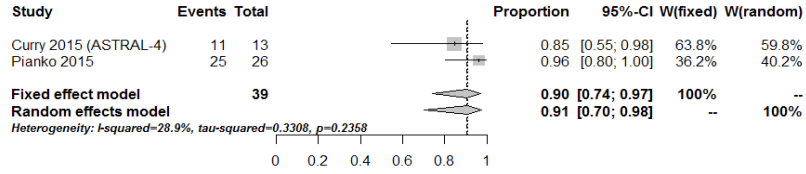
SOF PR 12



SOF VEL(100mg) 12



SOF VEL(100mg) RBV 12



Supplementary File 6.

Excluded studies in sensitivity analyses:

a) Excluding high risk of bias studies: Foster (EAP), Gane (ELECTRON), Hézode (ATU), Lawitz (FISSION), Lawitz 2015, Lawitz 2015, Lin 2015, Molina (PHOTON-2), Nelson (ALLY-3), Poordad (ALLY-1), Shah 2016, Sulkowski (PHOTON-1), Welzel (CUP), Wyles (ALLY-2), Zeuzem (VALENCE)

b) Excluding studies with not approved regimens: Foster 2011, Dore (AI444-031), Lawitz 2015, Pianko (2015), Everson (2015)

c) Excluding studies with decompensated liver disease patients: Curry (ASTRAL-4), Foster (EAP), Lin 2015, Welzel (CUP), Hézode (ATU), Poordad (ALLY-1)

d) Excluding studies with HIV/HCV coinfected patients: Sulkowski (PHOTON-1), Molina (PHOTON-2), Wyles (ALLY-2), Welzel (CUP), Hézode (ATU), Foster (EAP)

Sensitivity analyses patients without cirrhosis

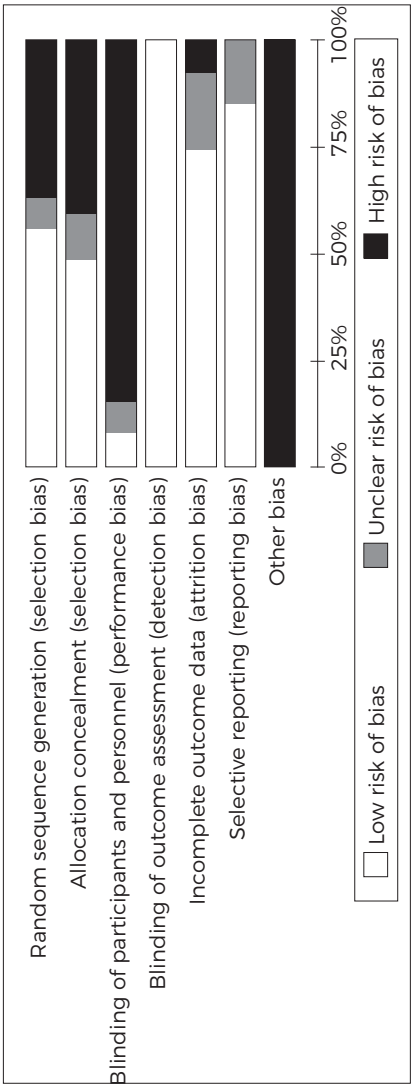
Regimen	Primary analysis including all studies	a) Analysis excluding high risk of bias studies	b) Analysis excluding not approved regimens	c) Analysis excluding studies with decompensated liver disease patients	d) Analysis excluding studies with HIV/HCV coinfected patients
SOF + DCV + RBV 24	98.9 (97.6-99.6)	96.8 (88.2-99.6)	98.9 (97.5-99.7)	99.3 (98.1-99.8)	98.8 (96.4-99.8)
SOF + VEL(100mg) + RBV 12	98.8 (97.5-99.6)	98.8 (97.0-99.7)	98.8 (96.9-99.7)	99.4 (98.7-99.8)	99.3 (98.2-99.8)
SOF + DCV + RBV 16	98.0 (95.7-99.2)	94.7 (80.8-99.4)	98.1 (95.7-99.4)	98.4 (95.9-99.6)	97.7 (93.4-99.5)
SOF + DCV 24	97.3 (94.2-99.0)	88.6 (63.0-98.5)	97.7 (94.6-99.3)	97.8 (94.2-99.4)	95.7 (87.4-99.1)
SOF + VEL(100mg) 12	97.2 (94.7-98.8)	95.3 (89.4-98.5)	97.5 (94.7-99.1)	98.3 (96.6-99.3)	97.4 (94.6-99.0)
SOF + DCV + RBV 12	95.6 (91.6-98.1)	91.0 (68.9-98.8)	95.3 (90.5-98.2)	95.9 (89.9-98.8)	95.0 (86.2-98.8)
SOF + PR 12	95.0 (90.6-97.8)	91.7 (80.8-97.3)	95.4 (90.9-98.1)	95.7 (92.1-98.1)	94.6 (89.3-97.9)
SOF + VEL(25mg) + RBV 12	94.3 (86.6-98.3)	94.7 (85.7-98.7)	Excluded	96.4 (91.3-99.1)	96.0 (89.5-99.0)
SOF + LDV + RBV 12	92.6 (84.9-97.2)	88.5 (55.9-99.0)	92.1 (83.2-97.4)	89.2 (67.5-98.3)	88.1 (54.7-99.0)
SOF + DCV 12	89.9 (80.9-95.6)	73.3 (33.1-95.2)	90.5 (80.4-96.3)	88.3 (73.5-96.1)	84.3 (61.8-95.7)
SOF + VEL(25mg) 12	87.4 (73.8-95.5)	82.0 (60.7-94.1)	Excluded	89.9 (79.2-96.5)	87.3 (72.2-96.0)
SOF + RBV 24	87.3 (80.5-92.4)	79.3 (62.1-90.8)	87.9 (80.3-93.5)	90.3 (85.2-94.4)	86.5 (77.6-93.1)
SOF + LDV 12	83.8 (68.7-93.5)	69.3 (22.1-95.9)	84.9 (68.6-94.6)	74.0 (36.6-94.5)	71.0 (23.7-96.5)
DCV + PR 16	81.2 (61.5-93.4)	71.5 (32.6-94.0)	Excluded	82.3 (64.1-93.6)	80.3 (58.7-93.6)
SOF + RBV 16	78.8 (67.8-87.3)	68.9 (46.6-85.2)	80.8 (69.2-89.7)	80.8 (71.4-88.4)	77.4 (63.9-87.7)
TPR + PR 24	70.2 (37.6-92.4)	64.5 (24.4-93.2)	Excluded	67.9 (36.2-91.0)	70.3 (35.9-93.2)
PR 24	67.6 (52.7-80.2)	56.4 (19.9-87.0)	67.9 (50.0-82.3)	65.9 (51.6-78.3)	65.6 (47.7-80.7)
DCV + PR 12	66.6 (40.4-86.6)	59.8 (20.9-89.4)	Excluded	63.7 (38.2-84.2)	64.7 (36.1-86.7)
SOF + RBV 12	62.5 (48.8-74.6)	55.4 (30.6-76.4)	61.9 (45.8-76.1)	60.8 (47.5-72.4)	60.0 (42.8-75.2)
OMB + PTV + RBV 12	39.1 (7.1-81.6)	Excluded	Excluded	41.1 (9.8-81.0)	42.7 (6.1-88.0)
SOF 12	41.1 (22.7-60.5)	24.7 (7.3-51.1)	44.3 (21.9-67.6)	33.4 (16.5-51.8)	30.2 (13.1-51.5)
OMB + PTV 12	23.3 (2.9-63.9)	Excluded	Excluded	20.4 (3.0-56.0)	20.9 (1.6-66.1)
OR RBV	2.6 (1.3-4.7)	4.6 (1.9-9.3)	2.3 (1.0-4.6)	3.4 (1.6-6.6)	3.9 (1.8-7.5)

Sensitivity analyses patients with cirrhosis

Regimen	Primary analysis including all studies	a) Analysis excluding high risk of bias studies	b) Analysis excluding not approved regimens	c) Analysis excluding studies with decompensated liver disease patients	d) Analysis excluding studies with HIV/HCV coinfected patients
SOF + VEL(100mg) 24	96.3 (92.0-98.7)	93.2 (83.2-98.4)	96.7 (92.3-99.1)	98.4 (96.2-99.5)	96.2 (91.0-98.9)
SOF + DCV + RBV 24	94.1 (86.8-98.1)	87.6 (58.7-98.7)	93.6 (84.4-98.3)	96.0 (88.8-99.2)	92.8 (79.3-98.7)
SOF + VEL(100mg) + RBV 12	93.7 (85.9-98.0)	94.6 (85.7-98.8)	92.9 (82.0-98.4)	96.8 (92.4-99.1)	95.4 (88.8-98.8)
SOF + DCV + RBV 16	89.8 (77.9-96.6)	81.0 (44.5-97.6)	89.6 (75.8-97.0)	91.8 (78.4-98.1)	87.7 (66.9-97.4)
SOF + DCV 24	87.3 (75.8-94.7)	67.0 (26.8-94.0)	88.1 (75.7-95.5)	88.9 (73.3-96.9)	79.5 (52.7-94.6)
SOF + VEL(100mg) 12	86.8 (77.4-93.6)	82.2 (66.5-93.2)	87.4 (76.7-94.8)	91.2 (84.2-96.1)	86.2 (75.1-94.1)
SOF + DCV + RBV 12	80.1 (62.7-92.0)	71.7 (29.8-95.7)	77.4 (56.1-91.9)	81.0 (57.9-94.6)	76.5 (47.7-93.9)
SOF + PR 12	77.8 (59.6-90.5)	71.6 (43.8-90.8)	77.8 (57.4-91.5)	79.7 (62.1-91.8)	74.5 (52.7-89.9)
SOF + VEL(25mg) + RBV 12	75.8 (50.4-92.5)	80.2 (52.8-95.3)	Excluded	83.1 (61.9-95.6)	80.1 (54.9-94.9)
SOF + LDV + RBV 12	70.3 (47.0-88.3)	67.7 (20.0-96.3)	67.4 (41.0-88.1)	63.3 (24.8-91.8)	61.5 (15.5-94.8)
SOF + DCV 12	63.3 (44.5-79.5)	43.0 (9.6-82.5)	63.2 (42.0-80.9)	59.4 (31.9-82.1)	50.1 (20.6-78.8)
SOF + VEL(25mg) 12	58.0 (34.1-79.6)	52.5 (25.6-78.2)	Excluded	62.9 (39.1-83.5)	55.3 (29.3-79.6)
SOF + RBV 24	56.3 (37.6-73.8)	47.3 (22.7-73.6)	55.8 (34.2-75.9)	62.7 (43.1-79.7)	52.0 (30.9-73.0)
SOF + LDV 12	50.6 (29.3-72.1)	39.6 (5.9-84.8)	51.0 (27.9-73.9)	38.7 (9.1-76.0)	36.1 (4.7-82.2)
DCV + PR 16	46.9 (20.5-74.9)	40.5 (8.9-80.4)	Excluded	47.6 (21.3-75.4)	43.0 (16.6-73.1)
SOF + RBV 16	41.6 (23.8-61.2)	34.7 (13.8-61.6)	43.0 (22.6-65.2)	43.8 (25.2-63.9)	37.2 (18.7-59.0)
DCV + PR 12	29.5 (9.8-57.9)	28.9 (5.0-68.7)	Excluded	26.2 (8.7-53.0)	25.9 (7.5-54.9)
PR 24	28.9 (14.2-47.9)	25.8 (4.7-63.9)	28.0 (11.2-50.1)	26.7 (12.8-45.3)	25.1 (10.7-45.4)
SOF + RBV 12	24.5 (12.2-40.7)	23.3 (7.5-47.7)	22.9 (9.9-41.2)	22.5 (11.0-38.3)	20.8 (8.9-38.1)
OR RBV	4.5 (2.5-7.7)	6.9 (3.0-14.2)	4.4 (2.1-8.3)	6.4 (2.7-13.3)	7.8 (3.6-15.4)

Supplementary File 7.

a) Risk of bias across studies



b) Risk of bias per study

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Curry 2015 (ASTRAL-4)	+	+	-	+	+	+	-
Dore 2015 (AI444-031)	+	+	?	+	+	+	-
Everson 2015	+	+	-	+	+	+	-
Foster 2011	+	+	-	+	+	+	-
Foster 2015 (ASTRAL-3)	+	+	-	+	+	+	-
Foster 2015 (BOSON)	+	+	-	+	+	+	-
Foster 2016 (EAP)	-	-	-	+	+	+	-
Gane 2013 (ELECTRON)	?	?	-	+	?	?	-
Gane 2015	+	+	-	+	+	+	-
Hézode 2015 (ATU)	?	-	?	+	-	?	-
Jacobson 2013 (FUSION)	+	+	+	+	+	+	-
Jacobson 2013 (POSITRON)	+	+	+	+	+	+	-
Lawitz 2013 (FISSION)	+	+	-	+	?	+	-
Lawitz 2015	-	-	-	+	+	+	-
Lawitz 2015*	-	-	-	+	+	+	-
Leroy 2016 (ALLY-3+)	+	+	-	+	+	+	-
Lin 2015*	-	-	-	+	?	+	-
Molina 2015* (PHOTON-2)	-	-	-	+	+	+	-
Nelson 2015* (ALLY-3)	-	-	-	+	+	+	-
Pianko 2015	+	+	-	+	+	+	-
Poordad 2015 (ALLY-1)	-	-	-	+	?	?	-
Shah 2016	+	?	-	+	?	+	-
Sulkowski 2014 (AI444040)	+	+	-	+	+	+	-
Sulkowski 2014 (PHOTON-1)	-	-	-	+	+	+	-
Welzel 2015 (CUP)	-	-	-	+	-	?	-
Wyles 2015 (ALLY-2)	+	?	-	+	+	+	-
Zeuzem 2014 (VALENCE)	-	-	-	+	+	+	-

These graphs shows risk of bias on 7 components across studies (a) and per study (b). Risk of bias was assessed with the Cochrane risk of bias tool

Supplementary File 8.

Analysis with treatment naive and treatment experienced patients

Treatment naive patients

Regimen	Estimated SVR (95% CrI)
SOF + DCV + RBV 24	98.3 (96.2-99.5)
SOF + VEL(100mg) + RBV 12	98.8 (97.3-99.6)
SOF + DCV + RBV 16	97.2 (93.4-99.1)
SOF + DCV 24	94.2 (86.9-98.1)
SOF + VEL(100mg) 12	95.8 (91.6-98.3)
SOF + DCV + RBV 12	93.1 (85.7-97.5)
SOF + PR 12	94.4 (88.6-97.8)
SOF + LDV + RBV 12	88.9 (75.9-96.4)
SOF + DCV 12	78.6 (60.5-90.9)
SOF + RBV 24	86.0 (76.5-92.7)
SOF + LDV 12	68.8 (44.9-86.9)
DCV + PR 16	86.8 (69.9-96.0)
SOF + RBV 16	77.6 (63.7-88.0)
TPR + PR 24	70.0 (34.7-93.4)
PR 24	63.4 (45.1-79.1)
DCV + PR 12	72.5 (46.2-90.7)
SOF + RBV 12	57. (40.7-73.4)
OMB + PTV + RBV 12	43.5 (5.4-90.2)
SOF 12	27.4 (12.8-46.1)
OMB + PTV 12	21.4 (1.4-70.0)
SOF + VEL(25mg) 12	83.3 (64.7-94.4)

Treatment experienced patients

Regimen	Estimated SVR (95% CrI)
SOF + VEL(100mg) 24	98.1 (95.4-99.5)
SOF + DCV + RBV 24	96.9 (92.0-99.2)
SOF + VEL(100mg) + RBV 12	97.8 (94.5-99.4)
SOF + DCV + RBV 16	94.7 (86.5-98.6)
SOF + DCV 24	89.6 (76.5-96.7)
SOF + VEL(100mg) 12	92.4 (84.7-97.1)
SOF + DCV + RBV 12	87.8 (72.7-96.2)
SOF + PR 12	89.9 (77.8-96.7)
SOF + LDV + RBV 12	81.1 (58.7-94.4)
SOF + DCV 12	66.8 (42.5-85.6)
SOF + RBV 24	76.6 (58.2-89.6)
SOF + LDV 12	55.2 (28.4-79.8)
SOF + RBV 16	65.2 (43.1-83.1)
SOF + RBV 12	43.1 (22.7-65.3)
SOF + VEL(25mg) + RBV 12	90.6 (75.5-97.8)
SOF + VEL(25mg) 12	73.5 (48.4-90.5)

Dutch guidance for the treatment of chronic hepatitis C virus infection in a new therapeutic era

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Abstract

Background

A new era for the treatment of chronic hepatitis C is about to transpire. With the introduction of the first-generation protease inhibitors the efficacy of hepatitis C treatment improved significantly. Since then, the therapeutic agenda has moved further forward with the recent approval of sofosbuvir and the expected approval of agents such as simeprevir and daclatasvir. This paper, developed parallel to the approval of sofosbuvir, is to serve as guidance for the therapeutic management of chronic hepatitis C.

Methods

We performed a formal search through Pubmed, Web of science and ClinicalTrials.gov to identify all clinical trials that have been conducted with EMA-approved new agents in hepatitis C, for this version (April 2014) we focused on sofosbuvir. For each disease category, the evidence was reviewed and recommendations are based on GRADE.

Results

We identified 11 clinical trials with sofosbuvir and for each disease category recommendations for treatment are done. Not all disease categories were studied extensively and therefore in some cases we were unable to provide recommendations.

Conclusion

The recent approval of sofosbuvir will most likely change the therapeutic landscape of chronic hepatitis C. The use of sofosbuvir containing regimens can shorten the duration of therapy, increase efficacy and result in less side effects, compared to standard of care. The efficacy relative to the standard of care needs to be weighed against increased costs of sofosbuvir. With future approval of other direct acting antivirals, the outcome of hepatitis C treatment will likely improve further and this guidance will be updated.

Introduction

The recent approval of sofosbuvir (NS5B polymerase inhibitor) and the expected approval of other direct-acting antivirals (DAAs) such as simeprevir (protease inhibitor, PI) and daclatasvir (NS5A inhibitor) will change the therapeutic arena for chronic hepatitis C.[1] Until 2012 the treatment of chronic hepatitis C consisted of pegylated interferon with ribavirin (PR) for 24 to 48 weeks.[2] As of April 2012 two first-generation protease inhibitors, telaprevir and boceprevir, were approved for reimbursement in the Netherlands for patients infected with hepatitis C virus (HCV) genotype 1.[3] These agents improved efficacy but their safety profile was poor especially in cirrhotics.[3-6]
In the Netherlands, the estimated hepatitis C seroprevalence is 0.1 – 0.4%, and the highest prevalence is seen in first-generation migrants from HCV-endemic countries.[7-9] Approximately 50% of Dutch patients are infected with genotype 1, 30% with genotype 3 and 10% with both genotype 2 and 4.[10]

Sofosbuvir can be regarded as a game changer, it is an orally administered nucleotide polymerase inhibitor, has pangenotypic activity in vivo, a high barrier to resistance and an acceptable safety profile.[1, 11] Approval of other drugs in different classes of DAAs may be expected, first of all simeprevir (during revision approved) and daclatasvir. Additional drugs belonging to the protease inhibitor class (asunaprevir, ABT -450/r), the NS5A class (ledipasvir, ombitasvir) and the non nucleoside polymerase inhibitor class (dasabuvir) are in later stages of clinical development.[1]

This paper may serve as a current guidance for the therapeutic management of chronic hepatitis C. This update of the earlier guideline is necessary given the wealth of new information that has become available since.[3] As a static version will become outdated, we encourage to review the most current version on the websites of Netherlands association of hepato-gastroenterologists (NVMDL) or Netherlands association of internal medicine (NIV).[12]

Methods

We performed a formal search through the databases Pubmed, Web of Science and ClinicalTrials.gov to identify all relevant clinical trials performed with sofosbuvir, peginterferon and/or ribavirin for this version (April 2014). In addition we searched for future therapies and for the product characteristics provided by the FDA and EMA. Opinions, letters, narrative reviews, pre-clinical studies and articles in another language than English, Dutch or German were excluded. The search string is attached in Supplementary File 1. We limited the search for patients with HCV mono-infection. For each disease category (treatment naive, treatment experienced and cirrhotic patients) the evidence was reviewed by the first and second author. The treatment experienced

category consists of patients with a prior relapse, prior partial response or prior null response. Sustained virological response (SVR) is defined as an HCV RNA below the lower limit of quantification at 12 weeks after the end of treatment. We listed the results of all individual trials in tables according to disease category. The level of evidence was formulated based on the GRADE method with the quality of evidence and a strength of recommendation (Supplementary File 2).[13] The recommendations in this paper went through a formal approval process and was vetted by individual experts and all members of the NVMDL and representatives of the NIV.

Results

We formulated recommendations on basis of the available evidence and information from the label of sofosbuvir. The recommendations are given for each disease category. In case no recommendation is given, treatment can be deferred or we refer to the earlier guideline.[3] First, all currently approved agents and expected agents are listed, followed by recommended treatment options for the different HCV genotypes once sofosbuvir is approved. Recommendations are valid for all patients with an indication for treatment as stipulated by the earlier guideline.[3]

List of currently approved drugs for treating chronic HCV infection:

- Peginterferon: polyethylene glycol attached to interferon-α
    - o Peginterferon α-2a:180µg/week
    - o Peginterferon α-2b:1.5µg/kg/week
- Ribavirin: nucleoside analogue, weight based dose
  (< 75 kg 1000 mg/day and ≥ 75 kg 1200 mg/day, BID)
- Protease inhibitors (-previr):
    - o Simeprevir (during revision approved, will be included in updated version)
    - o Telaprevir: 2250 mg/day, BID or TID
    - o Boceprevir: 2400mg/day,TID
- Nucleotide polymerase inhibitor (-buvir):
    - o Sofosbuvir: 400mg/day, QD

List of HCV drugs in development:

This list is not exhaustive and can be expanded, we aimed to include drugs that are in phase III development.[1]

- Protease inhibitors (-previr):
    - o Asunaprevir
    - o Faldaprevir
    - o ABT-450/r (ritonavir-boosted)
    - o MK-5172

- NS5A inhibitors (-asvir):
    - o Daclatasvir
    - o Ledipasvir
    - o Ombitasvir (ABT-267)
    - o MK-8742
- Non nucleoside polymerase inhibitors (-buvir):
    - o Dasabuvir (ABT-333)

Watchful waiting

Watchful waiting is a preferable strategy in patients with no urgent indication for treatment based on the earlier guideline, in patients where no recommendation is given or when the quality of evidence is low and the strength of recommendation is weak (Level: C2).[3] There are several arguments in favor of this strategy: (A) not all patient groups are represented in clinical trials, therefore the evidence for recommendations is weak in certain disease categories, (B) with the introduction of sofosbuvir we still need pegylated interferon and ribavirin in many patients and (C) improved efficacy and reduced toxicity is expected from interferon-free combinations of DAAs likely to be approved in the near future.[1]

Recommendations by HCV genotype, disease stage and treatment history:

Genotype 1 treatment naive patients:

Recommendation: Sofosbuvir, peginterferon and weight-based ribavirin for 12 weeks (Level: B1)

Several trials have been performed in genotype 1 treatment naive patients (Table 1). The recommended therapy has been studied in two trials: NEUTRINO and ATOMIC. The NEUTRINO trial was a single group open label trial that achieved 89% SVR.[14] Patients without cirrhosis obtained 90% SVR in the ATOMIC trial. There was no additional benefit (i.e. no difference in SVR) of extension of treatment to 24 weeks or by extension with sofosbuvir monotherapy or sofosbuvir and ribavirin (n= 264).[15] The dose of sofosbuvir was determined on basis of the PROTON study where 200 and 400 mg of sofosbuvir were compared. Here the SVR rate was irrespective of the dose of sofosbuvir, however three patients in the 200 mg group had a viral breakthrough, hence the selection of 400 mg.[16] Only one trial was of high quality, other trials were open label trials of low to moderate quality.[13, 16]

Genotype 1 treatment experienced patients:

Recommendation: No recommendation based on data

The ELECTRON trial was the only trial that included treatment experienced genotype 1 patients, these patients received sofosbuvir with ribavirin (12 weeks), only one of ten patients achieved SVR.[17] The label recommends consideration of treatment with sofosbuvir, peginterferon and ribavirin for 12 weeks or extension to 24 weeks, but in our opinion more data are needed.[18]

Genotype 1 cirrhotic patients:

Recommendation: Watchful waiting (Level: C1)

Two clinical trials included patients with cirrhosis, the NEUTRINO trial reached 80% SVR with sofosbuvir on top of PR and 3 of 6 cirrhotic patients with unfavorable characteristics achieved SVR with sofosbuvir and ribavirin in a single centre trial.[14, 19] The quality of evidence for sofosbuvir is low, the toxicity of previous standard of care in cirrhotic patients is high and future agents (e.g. simeprevir) are promising, hence watchful waiting is recommended.[4]

Future perspective:

For genotype 1 patients, multiple trials are currently underway, promising agents are simeprevir, asunaprevir, ABT-450/r (protease inhibitors), daclatasvir, ledipasvir, ombitasvir (NS5A inhibitors) and dasabuvir (non nucleoside polymerase inhibitor). All oral treatment is expected to become possible in the near future for both treatment naive and treatment experienced patients. Simeprevir and sofosbuvir with or without ribavirin were studied in the COSMOS trial in two cohorts, in prior null-responders with F0-2 fibrosis (cohort 1) and in treatment naive or prior null responders with F3-4 fibrosis (cohort 2). High SVR rates were seen in cohort 1: 91-100% and in cohort 2: 94-96%.[20, 21, 22] Therefore the combined treatment of simeprevir and sofosbuvir can be a reasonable option for these categories of patients in the near future. Simeprevir with PR has been studied in the ASPIRE, PILLAR and PROMISE studies and high SVR rates of 70-85% are seen in cirrhotic patients with prior relapse or prior partial response.[23-25] Clinical trials with simeprevir have shown that a Q80K mutation in genotype 1a patients significantly reduces efficacy of the treatment.[26]

Sofosbuvir plus daclatasvir with or without ribavirin for 12 or 24 weeks has been studied in the AI444040 study, 126 treatment naive genotype 1 patients achieved 98% SVR. Furthermore 41 patients who failed therapy with telaprevir or boceprevir had 98% SVR with 24 weeks of sofosbuvir and daclatasvir with or without ribavirin. Cirrhotic patients were excluded.[27] Currently there is a compassionate use program of sofosbuvir and daclatasvir with or without ribavirin for Child-Pugh C patients available.

The combination of an NS5B polymerase inhibitor and an NS5A inhibitor are also studied in the LONESTAR, ION-1, ION-2 and ION-3 study. The LONESTAR is a single centre open label study in genotype 1 treatment naive patients and patients with virological failure on protease inhibitors. An SVR of 95-100% (n=100) with different regimens (i.e. sofosbuvir/ledipasvir with or without ribavirin, 8 or 12 weeks) was reached.[28] In the ION-1 and ION-2 trial SVR was reached in 94-98% of the patients with 12 weeks of sofosbuvir/ledipasvir with or without ribavirin.[29, 30] In the ION-3 trial treatment naive non-cirrhotic patients achieved 94% SVR with 8 weeks of sofosbuvir/ledipasvir.[31] Phase 2a trials have been performed with daclatasvir and asunaprevir in combination with PR or the non nucleoside polymerase inhibitor BMS-791325 in prior null responders and treatment naive patients for 12-24 weeks. High SVR rates, 92-100%, were achieved.[32-34] Three studies (n=571, n=297 and n=473) evaluated multiple regimens with ABT- 450/r, dasabuvir and ombitasvir with or without ribavirin in different combinations and durations. High SVR rates (83%-97%) were seen in treatment naive and treatment experienced non-cirrhotic patients.[35-37] The TURQUOISE-II trial studied the same regimen (with ribavirin) in compensated cirrhotics for 12 (n= 208) and 24 (n=172) weeks. SVR was achieved in respectively 92% and 96% of the patients.[38]

Table 1. Trials in HCV genotype 1 patients

Trial	Regime (weeks) 0 4 8 12 24 // 48	n	SVR	SVR (95% CI) 0 50 100	QoE
Genotype 1, treatment naive					
PROTON	SOF(200)+PR / PR / PR	48	90%		A
	SOF(400)+PR / PR / PR	47	91%		A
	Placebo+PR / PR	26	58%		A
NEUTRINO	SOF+PR	292	89%		C
ELECTRON	SOF+RBV	25	84%		C
ATOMIC	SOF+PR	52	90%		B
	SOF+PR	109	93%		B
	SOF+PR / SOF(+RBV)	155	91%		B
Osinusi et al^	SOF+RBV(wb)	10	90%		C
	SOF+RBV(wb)	25	68%		C
	SOF+RBV(600)	25	48%		C
Genotype 1, treatment experienced					
ELECTRON	SOF+RBV	10	10%		C
Genotype 1, cirrhosis					
NEUTRINO	SOF+PR	54#	80%		C
Osinusi et al* ^	SOF+RBV(wb)	6#	50%		C
	SOF+RBV(600)	7#	29%		C

PR = pegylated interferon with ribavirin; QoE: Quality of Evidence (A: high, B: moderate, C:low); RBV = ribavirin; SOF = sofosbuvir; SVR = sustained virological response; wb = weight based; * calculated 95% CI; ^ first cohort early-moderate fibrosis; second and third cohort unfavorable characteristics; # in cirrhotics: treatment naive

Genotype 2 treatment naive patients:

Recommendation: sofosbuvir and weight-based ribavirin for 12 weeks (Level: A1)

Patients with an HCV genotype 2 infection have an SVR rate of 74-83% with PR for 24 weeks[3, 39, 40] Multiple trials with sofosbuvir have been performed in treatment naive genotype 2 patients (Table 2). Two trials of high quality and one of low quality studied the recommended interferon-free regimen (POSITRON, FISSION and VALENCE) with consistent good results. The POSITRON trial included patients for whom interferon was not an option and reached 93% SVR irrespective of cirrhosis.[11] In the FISSION trial SVR was reached in 97% of patients, while in patients treated with peginterferon and ribavirin (800 mg) for 24 weeks SVR was achieved in 78%.[14] The results of the VALENCE trial are similar to FISSION and POSITRON for the recommended regimen.[41, 42] Addition of peginterferon showed no improved SVR rates.[16, 17] In conclusion, sofosbuvir with ribavirin for 12 weeks in genotype 2 patients was effective in high quality trials with implications for clinical practice because of an interferon free regimen with a shorter treatment duration than previous standard of care.[3]

Genotype 2 treatment experienced patients:

Recommendation: sofosbuvir and weight-based ribavirin for 12 weeks (Level: B1)

In the FUSION trial, genotype 2 patients were treated with either 12 or 16 weeks of sofosbuvir and ribavirin. Patients in the 12 weeks arm received four weeks of placebo, they reached 86% SVR and in the 16 weeks arm this was 94%. For non-cirrhotic patients the FUSION trial failed to demonstrate additional value of extending the treatment to 16 weeks, hence the recommendation of 12 weeks.[11] The POSITRON included 17 patients with unacceptable side effects in prior treatment and they achieved an SVR of 78% with sofosbuvir and ribavirin.[11] The results of the VALENCE trial demonstrated a 90% SVR with the recommended regimen.[18, 42] In another trial there was no additional value of peginterferon.[43] Again this treatment has significant implications for clinical practice because of the high SVR rates without interferon and shorter treatment duration. The trials were of high and low quality with consistent results.[11, 41, 43]

Table 2. Trials in HCV Genotype 2 patients

Trial	Regime (weeks) 0 4 8 12 24 // 48	n	SVR	SVR (95% CI) 0 50 100	QoE
Genotype 2, treatment naive					
POSITRON	SOF+RBV	109	93%		A
	Placebo	34	0%		A
FISSION	SOF+RBV	70	97%		A
	PR (RBV_{800})	67	78%		A
PROTON	SOF+PR	25[$]	92%		B
ELECTRON	SOF+(P)R	40[$]	100%		B
	SOF+PR	10[$]	100%		B
	SOF	10[$]	60%		B
VALENCE	SOF+RBV	32	97%		C
Genotype 2, treatment experienced					
FUSION	SOF+RBV	36	86%		A
	SOF+RBV	32	94%		A
POSITRON	SOF+RBV	17[$]	77%		A
	Placebo	8[$]	0%		A
VALENCE	SOF+RBV	41	90%		C
LONESTAR-2*	SOF+PR	23	96%		C
Genotype 2, cirrhosis					
POSITRON*	SOF+RBV	17[#]	94%		A
	Placebo	13[$#]	0%		A
FISSION	SOF+RBV	49[$#]	47%		A
	PR (RBV_{800})	50[$#]	38%		A
VALENCE	SOF+RBV	2[#]	100%		C
		9[&]	78%		C
FUSION	SOF+RBV	10[&]	60%		A
	SOF+RBV	9[&]	78%		A
LONESTAR-2*	SOF+PR	14[&]	93%		C

PR = pegylated interferon with ribavirin; QoE: Quality of Evidence (A: high, B: moderate, C:low); RBV = ribavirin; SOF = sofosbuvir; SVR = sustained virological response; *calculated 95% CI; [$] data of genotype 2 and 3 combined; In cirrhotics: [#] treatment naive, [&] treatment experienced

Genotype 2 cirrhotic patients:

Recommendation: sofosbuvir and weight-based ribavirin for 12 weeks (Level: B1)

There are four trials that evaluated sofosbuvir and ribavirin for 12 weeks in cirrhotic genotype 2 patients, mainly treatment naive patients were studied. The FISSION demonstrated an SVR of 83% (n=12), treatment with peginterferon and ribavirin (800 mg) for 24 weeks led to 62% SVR (n=13).[14, 18] The POSITRON trial showed an SVR of 94%. In treatment-experienced patients with cirrhosis an extension of duration of treatment from 12 to 16 weeks led to an improvement in SVR from 60% (n= 10) to 78% (n= 9) in the FUSION trial.[11] The VALENCE trial shows 82% SVR in 11 cirrhotic patients with sofosbuvir and ribavirin (12 weeks).[18, 44] All trials included only small numbers of patients, but implications for clinical practice are high as treatment is warranted and toxicity is expected to be less than standard of care.

Future perspective

For genotype 2 patients the regimen of sofosbuvir with ribavirin leads to high SVR rates. Also, the AI444040 trial studied 26 treatment naive genotype 2 patients, 24 (92%) achieved SVR with different regimens consisting of sofosbuvir and daclatasvir with or without ribavirin for 24 weeks. Cirrhotic patients were excluded.[27]

Genotype 3 treatment naive patients:

Recommendation:

- *o watchful waiting*
- *o peginterferon and ribavirin (800mg) for 24 weeks*
- *o sofosbuvir and weight-based ribavirin for 24 weeks*
- *o sofosbuvir, peginterferon and weight-based ribavirin for 12 weeks*

(Level A2)

For genotype 3 patients, several options for treatment are available and the physician has to decide which strategy is currently better for the individual patient. Historically genotype 2 and genotype 3 patients achieve an SVR of 70-80% with peginterferon and ribavirin (800 mg) for 24 weeks.[3]

Different trials have been performed in genotype 3 patients, all trials with 12 weeks of sofosbuvir and ribavirin fail to show superiority in comparison with PR treatment (Table 3).[14] The addition of peginterferon or extension of treatment to 24 weeks showed improved results. In the ELECTRON trial, 25 patients received 12 weeks of sofosbuvir and ribavirin combined with peginterferon for 0, 4, 8 or 12 weeks, all patients achieved SVR.[17] The VALENCE trial obtained 94% SVR in 105 patients with sofosbuvir with ribavirin for 24 weeks.[18, 42] Because of above mentioned results peginterferon with ribavirin (800 mg) for 24 weeks remains an option for therapy, ribavirin should be weight-based in patients

with baseline characteristics associated with a poor response.[3] Other options are watchful waiting, sofosbuvir with ribavirin for 24 weeks or sofosbuvir with PR for 12 weeks. The choice for one of the regimens is dependent on the individual patient, bearing in mind the higher costs of sofosbuvir.

Genotype 3 treatment experienced patients:

Recommendation: Watchful waiting

Alternative strategy: sofosbuvir and weight-based ribavirin for 24 weeks OR sofosbuvir, peginterferon and weight-based ribavirin for 12 weeks (Level: B2)

Results of sofosbuvir for treatment experienced genotype 3 patients are disappointing with high imprecision, only the VALENCE and LONESTAR-2 trials show acceptable results but are of low quality. The FUSION trial showed that extension of treatment with 4 weeks led to improvement of SVR.[11] Extension to 24 weeks was done in the VALENCE study and an SVR of 79% was achieved, while for the non-cirrhotics the SVR rate was 87%.[18, 42] The LONESTAR-2 trial showed an SVR of 83% in 24 patients treated with sofosbuvir and PR for 12 weeks.[43] In the near future more effective combinations of DAAs are expected. Therefore the general recommendation is watchful waiting. As an alternative strategy sofosbuvir with ribavirin for 24 weeks or sofosbuvir with PR for 12 weeks may be considered.

Genotype 3 cirrhotic patients:

Recommendation: Watchful waiting

Alternative strategy: sofosbuvir and weight-based ribavirin for 16 weeks OR sofosbuvir and weight-based ribavirin for 24 weeks (Level: B2)

Genotype 3 cirrhotic patients were treated with sofosbuvir in five trials with moderate SVR rates. The FUSION trial showed an SVR of 19% with 12 weeks sofosbuvir and ribavirin in treatment experienced cirrhotic patients, extension of treatment to 16 weeks showed an SVR of 61%. The VALENCE trial studied 24 weeks of sofosbuvir and ribavirin in 60 cirrhotic patients, with 92% SVR in treatment naive patients and 62% in treatment experienced patients.[18] Based on above results with small numbers of patients we advise watchful waiting as the recommended strategy since SVR rates are rather low, mainly in treatment experienced patients and sofosbuvir is expensive. Alternative regimens are sofosbuvir and ribavirin for 16 weeks or 24 weeks.

Table 3. Trials in HCV Genotype 3 patients

Trial	Regime (weeks) 0 4 8 12 24 // 48	n	SVR	SVR (95% CI) 0 50 100	QoE
Genotype 3, treatment naive					
POSITRON	SOF+RBV	98	61%		A
	Placebo	37	0%		A
FISSION	SOF+RBV	183	56%		A
	PR (RBV_{800})	176	63%		A
PROTON	SOF+PR	25[$]	92%		B
ELECTRON	SOF+(P)R	40[$]	100%		B
	SOF+PR	10[$]	100%		B
	SOF	10[$]	60%		B
VALENCE	SOF+RBV	11	27%		C
	SOF+RBV	105	94%		C
Genotype 3, treatment experienced					
FUSION	SOF+RBV	64	30%		A
	SOF+RBV	63	62%		A
POSITRON	SOF+RBV	17[$]	77%		B
	Placebo	8[$]	0%		B
VALENCE	SOF+RBV	145	79%		C
LONESTAR-2*	SOF+PR	24	83%		C
Genotype 3, cirrhosis					
POSITRON*	SOF+RBV	14[#]	21%		A
	Placebo	13[$#]	0%		A
FISSION	SOF+RBV	49[$#]	47%		A
	PR (RBV_{800})	50[$#]	38%		A
VALENCE	SOF+RBV	13[#]	92%		C
		47[&]	62%		C
FUSION	SOF+RBV	26[&]	19%		A
	SOF+RBV	23[&]	61%		A
LONESTAR-2*	SOF+PR	12[&]	83%		C

PR = pegylated interferon with ribavirin; QoE: Quality of Evidence (A: high, B: moderate, C:low); RBV = ribavirin; SOF = sofosbuvir; SVR = sustained virological response; *calculated 95% CI; [$] data of genotype 2 and 3 combined; In cirrhotics: [#] treatment naive, [&] treatment experienced

Future perspective

Daclatasvir is one of the agents that is expected to be approved in the near future. The COMMAND GT 2/3 study included 151 genotype 2 and 3 patients and these patients received either 12 or 16 weeks daclatasvir with PR or 24 weeks placebo with PR. SVR rates were 69% (12 weeks), 67% (16 weeks) and 59% (placebo). Treatment failure was mainly due to relapse in cirrhotic patients in the 12 week group.[45] The combination of sofosbuvir and

daclatasvir with or without ribavirin for 24 weeks does hold promise for treatment naive genotype 3 patients as SVR rates of 89% can be reached.[27] Treatment naive genotype 3 patients received sofosbuvir/ledipasvir with or without ribavirin in the ELECTRON-2 trial (12 weeks). Dual therapy reached 64% SVR (n=25) while triple therapy reached 100% SVR (n=26).[46]

Genotype 4 treatment naive patients:

Recommendation: sofosbuvir, peginterferon and weight-based ribavirin for 12 weeks. (Level: C1)

The recommended regimen is studied in the NEUTRINO trial, 28 patients were treated with sofosbuvir and PR for 12 weeks and reached 96% SVR.[14] Extension of therapy to 24 weeks did not show improved effect.[15] Egyptian patients (n=28) received an interferon free regimen for 12 or 24 weeks and achieved 79% and 100% SVR respectively.[47] In general, data are scarce (Table 4) but in view of the high SVR rates sofosbuvir based treatment is recommended.

Genotype 4 treatment experienced patients:

Recommendation: No recommendation based on data

There are no published data of sofosbuvir based treatment available for treatment experienced genotype 4 patients. The most recent data of the Egyptian study showed 59% SVR (n=17) with 12 weeks sofosbuvir and ribavirin and 87% SVR (n=15) with 24 weeks sofosbuvir and ribavirin.[47, 48] The label recommends sofosbuvir and PR for 12 weeks, but more data are needed.

Genotype 4 cirrhotic patients:

Recommendation: No recommendation based on data

Only limited number of cirrhotic genotype 4 patients have been studied. The NEUTRINO trial included two cirrhotic genotype 4 patients of whom one achieved SVR with sofosbuvir and PR for 12 weeks.[14] In the Egyptian study treatment naive cirrhotic patients achieved 33% (n=3) and 100% (n=3) SVR with 12 and 24 weeks of sofosbuvir and ribavirin. The SVR rates in treatment experienced patients were 50% and 100% in both groups (n=8).[47]

Future perspective

Simeprevir with PR (24 or 48 weeks) is studied in genotype 4 patients, overall 65% of the patients reached SVR with higher SVR rates in treatment naive or relapse patients (83% and 86%).[49] Asunaprevir with PR has been studied in 18 genotype 4 patients for 24 weeks and 89% reached SVR, the control

group consisted of 7 patients of whom 43% reached SVR with PR for 48 weeks.[50] Furthermore daclatasvir was studied in 24 treatment naive genotype 4 patients, 67% achieved SVR with 20 mg daclatasvir and 100% achieved SVR with 60 mg daclatasvir with PR for 24 weeks.[51] Daclatasvir with asunaprevir and BMS-791325 were studied in 12 patients, 11 achieved SVR and 1 patient is still in follow-up.[52] The PEARL-I study included 86 treatment-naive genotype 4 patients who received ABT-450/r plus ombitasvir with or without ribavirin (12 weeks), 91-100% SVR was achieved.[53] Patient numbers are limited but in view of the high SVR rates of future therapy, watchful waiting can be considered in genotype 4 patients until further data allow approval of newer DAAs.

Genotype 5, 6

Data from well powered clinical comparative trials for genotype 5 and 6 patients are lacking. We think it is unlikely that such data will become available in the near future for the novel DAAs. Therefore we consider it acceptable to use treatment results for genotype 1 as a template for treatment of genotype 5 and 6.

Genotype 5, 6 treatment naive patients:

Recommendation:

- *Genotype 5: No recommendation based on data, consider genotype 1 treatment regimen as template (Level: C2)*
- *Genotype 6: sofosbuvir, peginterferon and weight-based ribavirin for 12 weeks (Level: C2)*

Only twelve treatment naive patients with genotype 5 or 6 have been treated in two trials (NEUTRINO and ATOMIC). In the NEUTRINO trial six genotype 6 patients and one genotype 5 patient have been treated with 12 weeks sofosbuvir and PR and all patients achieved SVR.[14] In the ATOMIC trial only five patients with genotype 6 received sofosbuvir with PR for 24 weeks, all achieved SVR.[15] More data is needed, however considering the high SVR rates a sofosbuvir based treatment is recommended for genotype 6.

Genotype 5,6 treatment experienced patients:

Recommendation: No recommendation based on data, consider genotype 1 treatment regimen as template (Level: C2)

There are no data of sofosbuvir based treatment available for treatment experienced genotype 5 or 6 patients.

Genotype 5, 6 cirrhotic patients:

Recommendation: No recommendation based on data, consider genotype 1 treatment regimen as template (Level: C2)

The NEUTRINO trial included 20% cirrhotic patients but it is unknown if cirrhotic genotype 5 or 6 patients were included.[14]

Table 4. Trials in HCV Genotype 4, 5 and 6 patients

Trial	Regime (weeks) 0 4 8 12 24 // 48	n	SVR	SVR (95% CI) 0 50 100	QoE
Genotype 4, treatment naive					
NEUTRINO	SOF+PR	28	96%		C
ATOMIC	SOF+PR	11	82%		C
Ruane et al*	SOF+RBV	14	79%		C
	SOF+RBV	14	100%		C
Genotype 4, treatment experienced					
Ruane et al*	SOF+RBV	17	59%		C
	SOF+RBV	15	87%		C
Genotype 4, cirrhosis					
NEUTRINO*	SOF+PR	2[#]	50%		C
Ruane et al*	SOF+RBV	3[#]	33%		C
		4[&]	50%		C
	SOF+RBV	3[#]	100%		C
		4[&]	100%		C
Genotype 5 and 6, treatment naive					
NEUTRINO*	SOF+PR	7	100%		C
ATOMIC	SOF+PR	5	100%		C
Genotype 5 and 6, treatment experienced					
No available trials					

PR = pegylated interferon with ribavirin; QoE: Quality of Evidence (A: high, B: moderate, C:low); RBV = ribavirin; SOF = sofosbuvir; SVR = sustained virological response; *calculated 95% CI, if 100% SVR then no CI could be calculated; In cirrhotics: [#] treatment naive, [&] treatment experienced

Drug-drug interactions

Many of the DAAs are substrates of CYP450 and the membrane transporter P-gp; they may both be the victim of drug interactions or cause these interactions with other agents.[54, 55] Sofosbuvir has a relatively mild drug interaction profile as it is only a substrate of P-gp and does not interfere with CYP450 enzymes. It is necessary to check for interacting co-medications, including over the counter drugs (e.g. St. John's worth), before starting DAA-based HCV treatment (http://www.hep-druginteractions.org).

Discussion

The current guidance comes at a time that the landscape of HCV treatment undergoes a rapid change. There are currently four comparable guidelines, one was issued by the AASLD, one by EASL and the other two are guidelines from Germany.[56-59] Our guidance differs from the AASLD and EASL guidelines and we do not offer advice on the use of simeprevir and daclatasvir in this version. The main difference with the other guidelines is that we offer the clinician the option to defer treatment in genotype 3 and some subgroups of patients. The main reason is that the currently published evidence, except for the VALENCE trial, has not proved efficacy beyond standard of care. The proportion of cirrhotics in the various trials is disappointingly low and recommendations cannot be given for this category, with the exception of genotype 2. This contrasts with clinical practice where cirrhotic patients have the most urgent treatment indication.[3]

For genotypes 5 and 6 the current evidence is poor. The AASLD, EASL and German guidances recommend sofosbuvir triple therapy for genotype 5 and 6. The consensus in the Hepatology committee was that the evidence for sofosbuvir was acceptable for genotype 6 naive patients, while we recommend standard of care or considering the genotype 1 regimen as template for other disease categories in genotype 5 and 6. At odds with other guidances we do not recommend sofosbuvir based treatment for genotype 1 and 4 treatment experienced patients given the lack of evidence. This guidance only includes recommendations for HCV monoinfected patients. Sofosbuvir and other DAAs are also studied in HIV/HCV patients, this will be updated in a new version of this guidance.

The rapid pace of development of drugs to treat HCV infection introduces not only great expectations but also uncertainty about the optimal timing to initiate therapy.[60] The key question here is which patients can benefit from the DAAs that are available now. Sofosbuvir is a first-generation polymerase inhibitor that is in the vanguard of a wave of drugs that have the potential to cure HCV. With the approval by EMA, sofosbuvir will be released on the Dutch market soon. As medication is an important costdriver, the added efficacy of sofosbuvir relative to standard of care should be weighed carefully.[61] As the pipeline with new antiviral drugs is full and new releases can be expected in 2014 and 2015, this paper serves as a dynamic document and will be continually edited and updated.[12]

Summary box of recommendations for HCV monoinfected patients:

Dosing for currently recommended agents:

- Sofosbuvir 400 mg once daily oral; there are no data in patients with renal impairment available (eGFR < 30 ml/min/1.73m^2)
- Peginterferon α-2a 180µg/week subcutaneous
  Peginterferon α-2b 1,5 µg/kg/week subcutaneous
- Ribavirin weight based: < 75 kg 1000 mg/day, ≥ 75 kg 1200 mg/day, BID

Geno type	Patient group	Recommendation	Future perspective
1	Treatment naive	Sofosbuvir, peginterferon and ribavirin for 12 weeks	Daclatasvir, simeprevir, ledipasvir, asunaprevir, ABT- 450/r, dasabuvir, ombitasvir
	Treatment experienced	No recommendation based on data	
	Cirrhotic	Watchful waiting	
2	Treatment naive	Sofosbuvir and ribavirin for 12 weeks	Daclatasvir
	Treatment experienced	Sofosbuvir and ribavirin for 12 weeks	
	Cirrhotic	Sofosbuvir and ribavirin for 12 weeks	
3	Treatment naive	Physician opinion to determine the strategy, options: - Watchful waiting - Peginterferon and ribavirin (800 mg) for 24 weeks - Sofosbuvir and ribavirin for 24 weeks - Sofosbuvir, peginterferon and ribavirin for 12 weeks	Daclatasvir, ledipasvir
	Treatment experienced	Watchful waiting Alternative strategy: Sofosbuvir and ribavirin for 24 weeks OR Sofosbuvir, peginterferon and ribavirin for 12 weeks	
	Cirrhotic	Watchful waiting Alternative strategy: Sofosbuvir and ribavirin for 16 weeks OR Sofosbuvir and ribavirin for 24 weeks	
4	Treatment naive	Sofosbuvir, peginterferon and ribavirin for 12 weeks	Simeprevir, daclatasvir, asunaprevir, ABT-450/r, ombitasvir
	Treatment experienced	No recommendation based on data	
	Cirrhotic	No recommendation based on data	
5,6	Treatment naive	Genotype 5: No recommendation based on data, consider genotype 1 treatment regimen as template Genotype 6: Sofosbuvir, peginterferon and ribavirin for 12 weeks	
	Treatment experienced	No recommendation based on data, consider genotype 1 treatment regimen as template	
	Cirrhotic	No recommendation based on data, consider genotype 1 treatment regimen as template	

Abbreviations

AASLD	American Association for the Study of Liver Diseases
BID	Bis In Die (twice a day)
CYP	CYtochrome P450
DAAs	Direct-Acting Antivirals
EASL	European Association for the Study of the Liver
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
HCV	Hepatitis C Virus
NIV	Netherlands Association of Internal Medicine
NVMDL	Netherlands Association of Hepato-gastroenterologists
PI	Protease Inhibitor
PR	Peginterferon and Ribavirin
RBV	Ribavirin
SOF	Sofosbuvir
SVR	Sustained Virological Response
QD	Quaque Die (once a day)
QoE	Quality of Evidence
TID	Ter In Die (three times a day)

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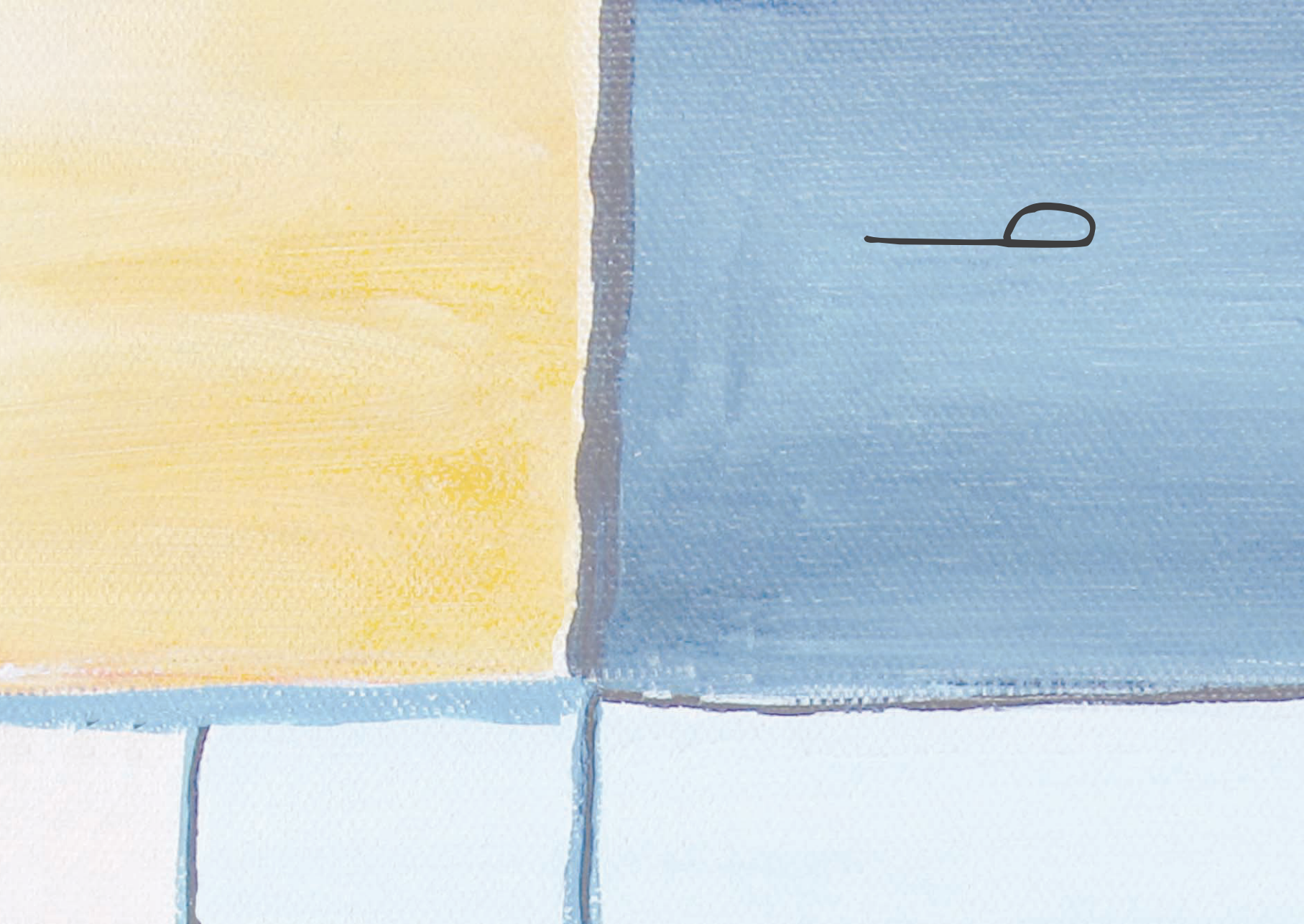
Supplementary Files

Supplementary File 1. Search

An initial search was conducted on 25-Feb-2014 with the term: '2-((5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-ylmethoxy) phenoxyphosphorylamino) propionic acid isopropyl ester [Supplementary Concept]' as a Mesh term. Furthermore we included 'sofosbuvir OR GS-7977 OR PSI 7977 OR PSI7977 OR PSI-7977 OR Sovaldi' in our search. In total 98 articles were found in Pubmed. All were scanned on title and abstract for inclusion. New results of the search were added until 8-Apr-14. For the future perspectives we searched the agents in phase III of clinical trials, including simeprevir (as Mesh combined with 'simeprevir OR TMC 435350 OR TMC435350 OR TMC-435350 OR Olysio OR TMC 435 OR TMC435 OR TMC-435', with the limit of clinical trials). We did the same for daclatasvir, ledipasvir, asunaprevir and the ABT formulations in Pubmed. Clinicaltrials.gov was used to get more information about the unpublished trials. Prior to submission, the abstracts of the International Liver Congres 2014 (49th annual meeting of the European Association for the Study of the Liver) were scanned and relevant studies were included in the future perspectives of the different genotypes.

Supplementary File 2. Evidence grading (adapted from the GRADE system)

Level	Evidence quality	Strenght of recommendation
A1	High	Strong
B1	Moderate	Strong
C1	Low	Strong
A2	High	Weak
B2	Moderate	Weak
C2	Low	Weak



General discussion

Adapted from
Nederlands Tijdschrift voor Geneeskunde, 2016 Oct;160(0):D735.

The main aim of this thesis was to improve translation of results from clinical trials with direct acting antivirals (DAAs) in chronic hepatitis C to clinical practice. In 2012, the first signs of a development paradox in first-generation DAAs for chronic hepatitis C virus (HCV) became apparent. HCV patients treated in real world conditions had unexpected high rates of serious adverse events (SAEs) compared to those included in registration trials.[1] These trials were the basis for approval of these agents by regulatory authorities and should have given us a comprehensive picture of safety and efficacy of these agents. This was clearly not the case, and this motivated us to study the reasons for this paradox in Dutch HCV patients. We initiated a nationwide retrospective registry of all patients treated with the first-generation DAAs, telaprevir or boceprevir (n=467). We studied the generalizability of trials as a first step in translation of evidence from trials to practice. Further, we interpreted evidence from trials by conducting a network meta-analysis and by developing a guidance for HCV treatment in the Netherlands.

Generalizability of trials to clinical practice

In **chapter 2** we demonstrated that generalizability of trials with first-generation DAAs was poor. Almost half of patients who were treated in clinical practice would have been excluded from registration trials. Excluded patients had significantly more SAEs when exposed to first-generation DAAs than eligible patients (27% vs. 11%, p<0.001). DAAs were less effective in excluded patients in case very strict exclusion criteria were applied. In contrast, the real world patients who were eligible for the trials had comparable results as obtained from registration trials. Thus, the results of trials were only generalizable to patients who completely fulfilled the eligibility criteria.[2]

We studied three problems that might arise as a result of limited generalizability: **chapter 3** and **chapter 4** are about adverse events and **chapter 6** about drug-drug interactions (DDIs). In the pegylated interferon era, research was focused on the risk of infections and bleeding episodes related to bone marrow toxicity of peginterferon.[3-6] In **chapter 3** we found that 31% of patients treated with peginterferon, ribavirin and telaprevir or boceprevir developed an infection during therapy and 7% was categorized as severe.[7] This is in line with infection rates found with dual (peginterferon and ribavirin) therapy but are higher than those reported in the registration trials of telaprevir and boceprevir (severe infections 1-3%).[8-12] The CUPIC cohort was the first to describe the increased risk of infections in clinical practice with protease inhibitors. Patients with an albumin <35g/dL and a platelet count ≤100 x10^9/L had the highest risk, which is similar to findings from our study.[13] Both albumin and platelet count were identified in **chapter 2** as important exclusion criteria affecting generalizability, so the exclusion of these patients from the registration trials probably was key to the lower infection rates. In the subsequent study (**chapter 4**) we showed that 22% of patients experienced a bleeding episode, but majority (>80%) of bleedings were mild and did not necessitate intervention. The incidence of bleedings in our cohort was higher than reported in other dual therapy cohorts, possibly related to the protease inhibitor.[5, 14] In contrast,

the registration trials did not report bleeding episodes, suggesting that the prevalence was <10%. However, as the majority of bleeding episodes were mild, lack of generalizability did not seem to be a problem here.

Another interesting topic is the risk for DDIs. None of the registration trials of telaprevir and boceprevir gave a risk assessment or indicated relevance of DDIs. An extensive review highlighted this issue and summarized the DDIs with > 60 frequently used co-medications in HCV.[15] In addition, we found in **chapter 2** that concomitant medication was the most important reason for exclusion from trials in clinical practice. Not only the first-generation DAAs carry a risk for DDIs. In **chapter 6** we predicted the risk on DDIs of concomitant medication and new generation DAAs with the help of the Liverpool database (www.hep-druginteractions.org). We found that 60% of all patients were at risk for a relevant DDI with one of the DAA regimens. Our results are in line with other cohorts and support the involvement of a pharmacist when initiating HCV treatment.[16, 17] DDIs are clinically important because they can lead to decreased effectiveness or increased toxicity of both DAA and the concomitant drug. It is essential that pharmaceutical companies clearly describe this limitation of generalizability not only in the label, but also in the papers.

In **chapter 2** we identified criteria which affected effectiveness and safety, i.e. hepatic decompensation and co-morbidity such as cardiac disease and anemia. It turned out that these criteria were similar to important predictors of response in large real world cohorts with new generation DAAs: history of (decompensated) cirrhosis, platelet count, total bilirubin, serum albumin, and hemoglobin.[18] Unfortunately, criteria concerning co-morbidity and co-medication are often poorly justified in drug intervention studies.[19] By improving justification of criteria, generalizability will likely increase as well. With this in mind we were interested in the generalizability of the new generation DAAs. In **chapter 5** we showed that the patients in registration trials resemble real world patients more and more over time, indicating that the gap between trials and practice is closing. This probably results in an increased generalizability, which is indeed corroborated by the comparable effectiveness and safety results from large real world cohorts.[18, 20] Although, to assess the eligibility rate of a trial, the full set of eligibility criteria should be readily available. Unfortunately this is often not the case.[21] We were able to find 34 of 43 protocols (79%) in **chapter 5**, mainly because journals required to publish the protocols. However, even some landmark trials, for example the COSMOS trial, did not reveal the complete eligibility criteria.[22] In addition, the global trial register (ClinicalTrials.gov) often does not report the full set of eligibility criteria as well.[23] In our experience, mainly criteria on co-morbidity, co-medication and laboratory values are lacking, while these criteria are equally important.

Interpretation of evidence in clinical practice

For interpretation of evidence in clinical practice is good generalization necessary, but also comparative effectiveness data to identify which treatment option is best for the patient. In the field of HCV only few head-to-head comparisons have been performed and often the comparative arm was not standard of care.[24] Physicians and guideline developers need comparative effectiveness data to justify choices and prioritize treatments, and this information is often lacking. Yet, there are some ways to interpret data in case no comparative effectiveness studies are executed, for example by performing a network meta-analysis.[25, 26] In **chapter 7** we adopted a network meta-analysis and identified that sofosbuvir/velpatasvir regimens achieved highest SVR rates in HCV genotype 3 patients. In addition we found that ribavirin significantly boosts SVR rates in all patients (OR 2.6-4.5).[27] This methodology can be applied in other fields. However, it should not discourage pharmaceutical companies or researchers to design comparative effectiveness trials.

The rapid changing therapeutic landscape in HCV led to the need of a guidance in the Netherlands.[28] **Chapter 8** is the first version of this Dutch guidance for treatment of HCV patients, developed after the approval of sofosbuvir.[29] After the publication there was an initiative to collaborate with several associations within the Netherlands for the guidance (NIV, NVHB, NVMDL, NVH and NVZA). Collectively we aimed to guide physicians practically regarding indication and therapy for HCV patients and strived for a uniform and high quality treatment (www.hcvrichtsnoer.nl). Instead of grading all the published papers ourselves we summarized recommendations from international guidelines, applied this to the Dutch situation, and updated the website already six times in 2015-2016 after important new data or DAA approval.[30-33]

Reflection

With this thesis we aimed to improve translation of trials to clinical practice in HCV. The difficulty was the rapidly changing therapeutic field from availability of (solely) peginterferon and ribavirin until 2012 to a comprehensive set of interferon-free DAA combinations in 2016. One of the strengths of this thesis is that we were able to set up a nationwide registry with data of patients treated with first-generation DAAs from 47 hospitals. Unfortunately, collection of data was time-consuming which led to publication of our study at the time that first-generation DAAs were not prescribed anymore in the Netherlands. The retrospective character of the registry was another limitation, although, we think this does not lead to relevant bias as HCV is a straight forward disease with standardized therapy regimens, outpatient visits and an objective outcome measure (SVR: HCV RNA negativity 12 or 24 weeks after treatment). In addition, there were already several competing large prospective registries worldwide (e.g. TRIO, HCV-TARGET, Veterans Affairs) which study classic research questions about effectiveness and safety of DAAs in real world patients.

By focusing on generalizability issues we identified an important niche in HCV research. We discovered general principles, relevant for the development of new DAAs. Furthermore, despite the fact that telaprevir and boceprevir are not prescribed in the Netherlands anymore, we believe the research is still relevant for countries prescribing interferon based therapy.

Another strength of this thesis is that we used a relative new technique to interpret data from trials to practice. By using the network meta-analysis we could establish the most optimal regimen, but also the role of ribavirin in HCV. This was important because it was not properly studied in trials and could not be assessed in registries (confounding by (contra)indication). We do encourage researchers to use this methodology, as it provides relevant data for guideline developers. Concerning our guidance, it is unique that it is supported by hepatologists, infectiologists, and pharmacists in the field. We believe it has contributed to a uniform and high quality therapy in the Netherlands. Of course, this guidance is no formal guideline and not all evidence was assessed by ourselves, which is a limitation. At this time, this was the most feasible manner to produce an up-to-date guidance. We would advise the Dutch guidance group to write a formal evidence-based guideline once the key developments in field have happened.

Future directions

Ideally, registration trials (phase 3 trials) should be perfectly generalizable to the real world, i.e. registration trials should include all subgroups of patients with mild to severe disease (probably in separate studies), with various co-morbidities and concomitant medications. Results obtained in these trials should be directly translatable to practice. We have shown that generalizability in HCV was hampered because of use of strict inclusion and exclusion criteria. However, this issue is not unique to HCV but is also seen in psoriasis, rheumatoid arthritis and inflammatory bowel disease.[34-36] Justification of each exclusion criterion is essential to limit the selection bias.[19]

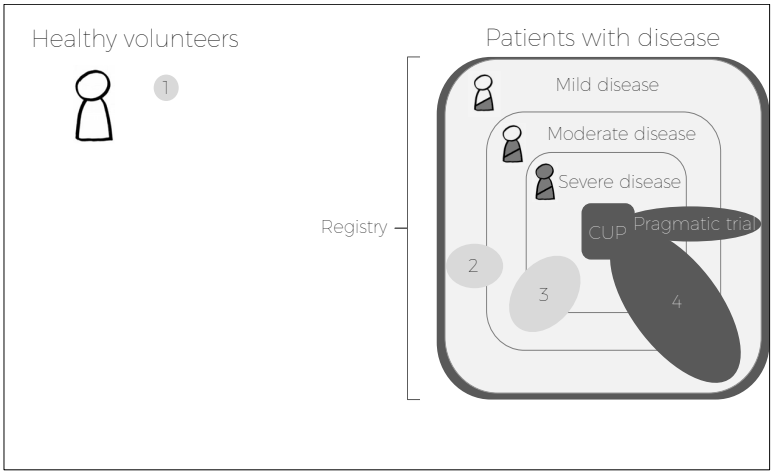
We do acknowledge that the outlined ideal situation is costly, time-consuming and often not feasible. However there are some ways to deal with the current shortcomings (visualized in Figure 1).[37] One option is to execute a pragmatic trial just before of after registration. In this trial there are no restrictions regarding inclusion or exclusion, but patients are randomized between the intervention and standard of care.[38] Further, we urge pharmaceutical companies to include specific subgroups of patients with severe disease in an early stage of research to assess the risks of therapy. Another option for these patients is a compassionate use or early access program, launched for adaptive licensing of drugs. Unfortunately, these programs are frequently unavailable.[39]

The responsibility to conduct trials properly lies with pharmaceutical companies and regulatory authorities. However, physicians can also contribute in clinical practice, for example by collecting data prospectively in well-designed registries. Especially when

drugs are very expensive, as is the case in HCV. There are some good examples such as the DREAM-registry (Dutch REumatoid Arthritis Monitoring) and the HIV-Monitoring database. It is paramount to develop an HCV-registry in the Netherlands. The data can be used to assess outcomes of treatment, behavior of physicians, but also long term effects of drugs. In addition, data from a registry could be used for the concept 'pay-for-performance', an arrangement where the payer gets reimbursed by the pharmaceutical company if the drug was not effective. Also data from registries can be combined with other data sources (evidence synthesis) to estimate treatment effects, identify clinically relevant predictors etcetera, for example by using Bayesian statistics.[40] Of note, data from a registry has the disadvantage of confounding by (contra)indication which emphasizes the importance of well-conducted (pragmatic) trials.[41]

In the case of HCV all of the above described options are possible to improve care. The ultimate goal is to eradicate HCV. To achieve this it is important that patients, including those who are unaware of the infection, are traced, treated and registered. Also, drugs should be cheaper and available worldwide. In the Netherlands we have the luxury to be able to prescribe drugs to all infected patients, in contrast to other countries where drugs are only reimbursed for patients with the highest need or not reimbursed at all.

Figure 1. Schematic visualization of population included in clinical trials and clinical practice



This figure shows which part of the population is included in various ways to collect data: 1 (phase 1 trial), 2 (phase 2 trial), 3 (phase 3 trial), 4, (phase 4 data), CUP (Compassionate Use Program), pragmatic trial, and a registry. The size represents the size of the trial or database. Light grey represents trials before registration and dark grey after registration; it is clear that not the whole disease spectrum is included in phase 1-2-3 research.

General conclusions

Generalization of data from registration trials to the clinical practice was limited for first-generation DAAs. Exclusion criteria related to predictors affected outcomes significantly and should be properly justified in future trials. Fortunately, trial patients do resemble real world patients more and more, especially when trials include subgroups of patients with severe disease (decompensated cirrhosis). A registry with real world data is paramount to assess (longterm) outcomes of HCV therapy. Further, data from both trials and registries can be combined by network meta-analysis for translation to practice and guidelines when comparative effectiveness data is lacking. However, conducting a pragmatic trial is a better option, in this way the quality of evidence improves which aids guideline developers in recommending therapy to physicians.

Abbreviations

DAAs	Direct-acting Antivirals
DDIs	Drug-Drug Interactions
DREAM	Dutch REumatoid Arthritis Monitoring
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
NIV	Netherlands Association of Internal Medicine
NVH	Netherlands Association of Hepatology
NVHB	Dutch Association of HIV-treating physicians
NVMDL	Netherlands Association of Hepato-gastroenterologists
NVZA	Netherlands Association of Hospital Pharmacists
SAEs	Serious Adverse Events

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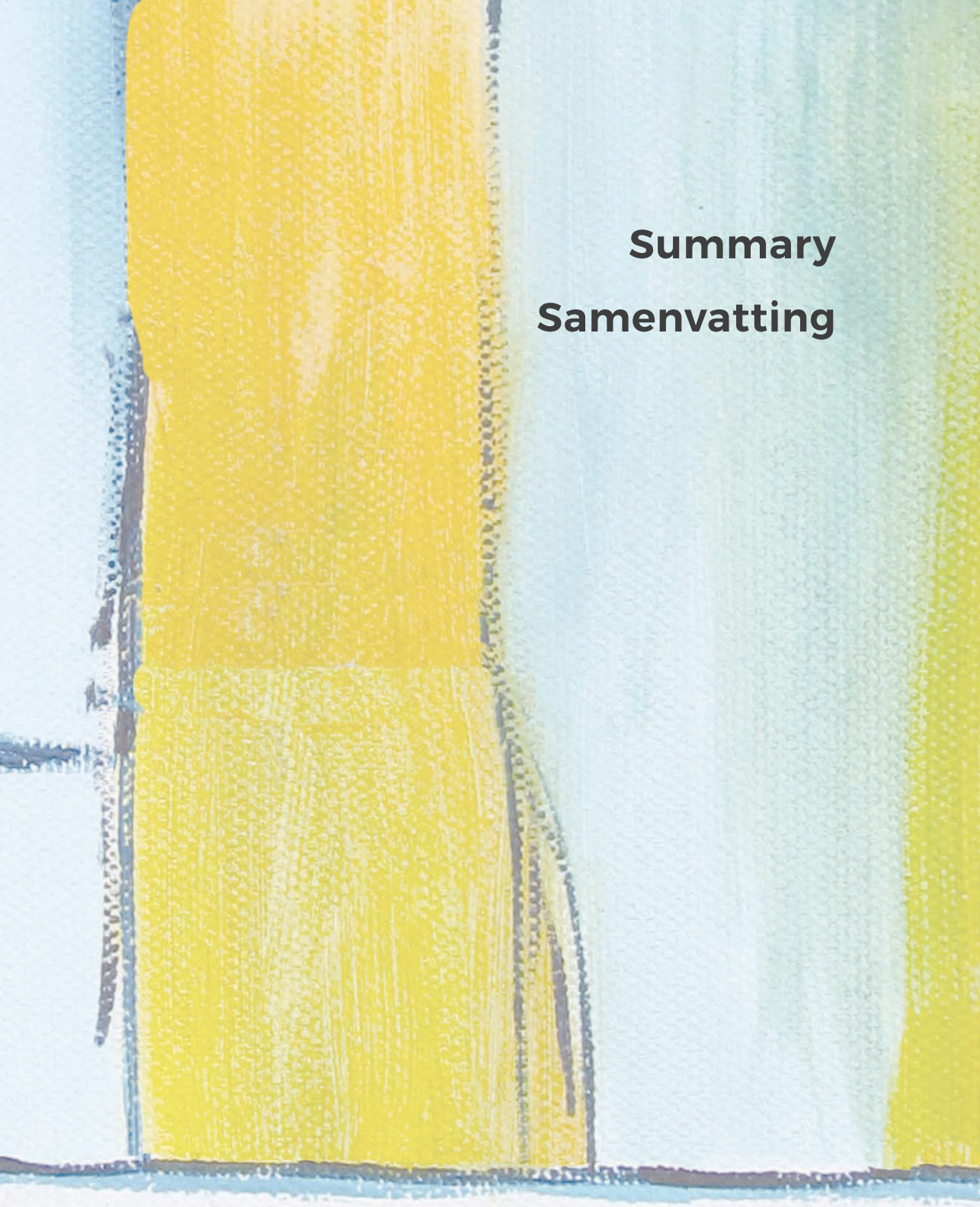

Summary

Samenvatting

Summary

Hepatitis C

Patients with a chronic hepatitis C virus (HCV) infection have contracted the hepatitis C virus and failed to spontaneously eradicate the virus. This RNA-virus can only be transmitted through blood-blood contact. Worldwide, there are about 130-150 million patients infected. There are seven different HCV genotypes identified, of which genotype 1 and 3 are most prevalent. Majority of HCV patients do not develop symptoms at infection, and only 20-25% of patients can clear the virus spontaneously. In case the virus is not cleared after 6 months, patients are classified as chronically infected. In this stage the liver is infected for months or years which can lead to scarring of the liver (fibrosis) and eventually cirrhosis (end-stage fibrosis). Progression to fibrosis and cirrhosis should be prevented as it can lead to development of hepatic decompensation and/or hepatocellular carcinoma.

Treatment of hepatitis C

Treatment has the goal to eradicate HCV. The surrogate marker for eradication is a sustained virological response (SVR), defined as HCV-RNA negativity 3-6 months after cessation of treatment. Until 2012 the treatment consisted of a combination of pegylated interferon (pegIFN) and ribavirin (RBV), but cure rates were disappointing (45-80%) and toxicity was high. Since 2012, the first direct-acting antivirals (DAAs) became available on the market in 3 classes: NS3/4A protease inhibitors, NS5A inhibitors and NS5B polymerase inhibitors. These new agents are combined and achieve high SVR rates (>90%) for patients with an HCV genotype 1, 2, or 4 infection. However, it is still a challenge to cure patients with HCV genotype 3.

Approval and registration of new drugs

In general, many trials have to be conducted to get approval of regulatory authorities for a drug. These trials are divided in three phases: phase 1, phase 2 and phase 3 trials. The latter are conducted to register the drug and are also called registration trials. These trials should be generalizable to clinical practice and should include a relevant comparator drug to apply the results to clinical practice. Both issues were not optimal in registration trials with DAAs for HCV.

This thesis focuses on the translation of HCV registration trials to clinical practice in two steps: 1) the generalization of trials conducted in patients with chronic hepatitis C and 2) the interpretation of evidence in clinical practice.

Generalizability of trials to clinical practice

In **chapter 2** we set up a nationwide retrospective registry of 467 Dutch HCV genotype 1 patients who were treated with telaprevir or boceprevir combined with pegIFN and RBV. We demonstrated that 47% of patients who were treated in clinical practice would have been excluded from registration trials with these first-generation DAAs. These patients had significantly more serious adverse events (SAEs) than patients who would have been included in the trials (27% vs. 11%, p<0.001). Effectiveness was reduced in case very strict eligibility criteria were applied. The real world patients who were eligible for the trials had comparable results as obtained from registration trials, indicating that results of trials are only generalizable to patients who fulfill the eligibility criteria. In our study we identified the eligibility criteria which affected effectiveness and safety, i.e. signs or history of hepatic decompensation (including low platelet count, low serum albumin and high bilirubin) and co-morbidity such as cardiac disease, malignancy, anemia and neutropenia. These criteria were similar to important predictors of response in a large real world cohort treated with new generation DAAs: history of (decompensated) cirrhosis, platelet count, total bilirubin, serum albumin, and hemoglobin. This suggests that the results of our study remain relevant in the new generation DAA era. Unfortunately, exclusion criteria are often not fully available in published papers. Even the global trial register (ClinicalTrials.gov) does not report full eligibility criteria. In our experience, mainly criteria on co-morbidity, co-medication and laboratory values are lacking. This hampers careful judgment of generalization of trials. Patients underrepresented in trials, but who receive treatment in clinical practice, can be exposed to suboptimal effectiveness or unforeseen harm.

In **chapter 3** and **chapter 4** we focused on two specific adverse events (infections and bleeding episodes) occurring in the cohort treated with first-generation protease inhibitors. These adverse events were already topic of research with pegIFN and RBV therapy, because bonemarrow suppression caused by pegIFN would increase the risk to develop infections and bleeding episodes. In **chapter 3** we found that 31% of 467 treated patients developed a clinically relevant infection during therapy and 7% was severe. The rates resemble infection rates found with dual therapy (pegIFN and RBV), but were higher than reported in the registration trials. We identified female sex, diabetes mellitus and chronic obstructive pulmonary syndrome as predictors for infection. Neutropenia was not associated with development of infections, indicating that advised dose reductions of pegIFN for neutropenia might be too strict, which can lead to reduced effectiveness. It is important to monitor the patients with risk factors carefully, rather than reduce the dose of pegIFN. In **chapter 4** we showed that 22% of patients treated with first-generation protease inhibitors developed a bleeding episode, but majority (>80%) of bleedings were mild and needed no intervention. We found ten severe bleedings: hematemesis (3), severe epistaxis (3), hemorrhagic cerebrovascular accident (2), rectal blood loss and a subdural hematoma.

The incidence of bleeding episodes in our cohort was higher than reported in other dual therapy cohorts, possibly induced by the protease inhibitor. Predictors for bleedings in our cohort were similar to risk factors with dual therapy: severe thrombocytopenia (<50 $x10^9$/L), female sex and cirrhosis. Still, as the majority of bleeding episodes were mild, the problem is often not clinically relevant.

The HCV therapies developed rapidly over time. In the past years several trials studied DAA regimens and current regimens are interferon-free. PegIFN had specific contra-indications and severe side effects which could limit inclusion of patients in trials, therefore we expected that eligibility criteria became less strict over time. In **chapter 5** we stated that the patients in registration trials resemble real world patients more and more over time. We studied this by calculating the proportion of patients from a clinical practice HCV cohort (n=177) who would be eligible for the registration trials (eligibility rate) of new DAA regimens. We found a linear association with start date of trials: the eligibility rate increased from on average 75% in 2008 to 89% in 2014. This probably resulted in an increased generalizability, which is supported by the comparable effectiveness and safety results from current large real world cohorts.

One might think that all problems with DAAs are resolved now, however in **chapter 2** we found that concomitant medication was the most important reason for exclusion from trials. The rational here is that DAAs inhibit/induce and can be substrates of drug-metabolizing enzymes and drug transporters. In **chapter 6** we predicted the risk on drug-drug interactions (DDIs) of concomitant medication and DAAs based on the Liverpool database (www.hep-druginteractions.org). We found that 77% of patients used concomitant medication and that 60% of all patients were at risk for a relevant DDI with one of the DAA regimens. DDIs are clinically important because they can lead to decreased effectiveness or increased toxicity of both the DAA and the concomitant drug. Antidepressants, protonpump inhibitors and benzodiazepine derivates were most frequently used, but also carry the risk of a DDI with a DAA. The newest generation DAA regimens (grazoprevir/elbasvir and sofosbuvir/velpatasvir) had the lowest DDI-potential while paritaprevir/ritonavir, ombitasvir and dasabuvir carried the highest risk. In total, 23% of concomitant medications (n=60) was not available in the database. We predicted that 11 of 60 drugs were at risk for potential DDIs when combined with DAAs. Our results are in line with other cohorts and encourage the involvement of a pharmacist when initiating HCV treatment.

Interpretation of evidence in clinical practice

Eventually all data obtained in phase 1, 2 and 3 trials are used for regulatory approval and for implementation in clinical practice. This stresses the importance of generalizability of trials. While the generalizability of DAA trials seemed to improve over time, the full interpretation of data is not optimal yet. One of the issues is comparative effectiveness. Only few head-to-head comparisons of DAAs were executed and the comparative

arm(s) usually lacked standard of care. Yet, there are some ways to interpret the data, for example by performing a network meta-analysis. A network meta-analysis is able to compare more than 2 treatments by combining direct and indirect evidence. Bayesian statistics can be used to estimate the treatment effect of each therapy. In **chapter 7** we identified that sofosbuvir/velpatasvir was the best DAA regimen for HCV genotype 3 patients with this method. We chose genotype 3 as efficacy of DAAs was suboptimal. Further, we found that ribavirin significantly increased SVR rates in all patients (odds ratio 2.6-4.5). These findings can aid guideline developers to prioritize treatment and physicians in their choice of therapy.

The Dutch guideline published in 2013 was outdated in 2014, hence an update was needed. **Chapter 8** is the first version of a Dutch guidance, developed after the approval of sofosbuvir. The speed of DAA development forced us to write a guidance instead of a formal guideline. We systematically reviewed all available evidence of sofosbuvir and formulated recommendations based on the GRADE method. After the publication, five Dutch associations collaborated for the future versions of the guidance: Netherlands Association of Internal Medicine (NIV), Dutch Association of HIV-treating physicians (NVHB), Netherlands Association of Hepato-gastroenterologists (NVMDL), Netherlands Association of Hepatology (NVH) and the Netherlands Association of Hospital Pharmacists (NVZA). The aim was to provide practical therapeutic guidance to physicians and to achieve high quality treatment in the Netherlands (www.hcvrichtsnoer.nl). We summarized recommendations from international guidelines and applied this to the Dutch situation. We updated the website regularly after important new data or DAA approval.

Conclusions and future directions

We have shown that strict eligibility criteria of HCV registration trials limited generalization of results, which led to unforeseen harm in clinical practice patients. We have identified general principles for improving generalizability: 1) justifying all eligibility criteria is needed with special attention for exclusion criteria related to predictors of response, this should stretch the eligibility rate to >95% and improve generalizability; 2) the full set of eligibility criteria of each trial should be available to adequately assess the generalizability of the trial; 3) we identified subgroups which should be studied prior to registration of a DAA to prevent unexpected harm in clinical practice (patients with hepatic decompensation and comorbidity such as anemia, malignancy and cardiac disease); and 4) we showed the need of involving a pharmacist in starting HCV treatment as the risk of DDIs is high and not appropriately mentioned in the publications of HCV trials. Fortunately, trial patients resemble clinical practice patients more and more. However, the introduction of pragmatic trials, trials with a relevant comparator, more accessible compassionate use programs and inclusion of patients with the highest need for therapy in an early phase of drug development could improve generalizability in the HCV field. In addition, a registry of treated HCV patients is

necessary to assess outcomes and long term effects of drugs. Data of above mentioned improvements can be synthesized to assess true treatment effects for clinical practice and can be used for guidelines. An example of this evidence synthesis is our network meta-analysis. Currently, our HCV guidance is still in use, however once the key developments have happened, it is time to write a formal guideline based on original data.

Samenvatting

Hepatitis C

Mensen met hepatitis C hebben een ontsteking van de lever die veroorzaakt wordt door het hepatitis C virus. Dit virus wordt verspreid door bloed-bloed contact, bijvoorbeeld via besmette bloedtransfusies of bloedproducten (vóór 1992) of via het delen van naalden bij drugsgebruik. Daarnaast komt de ziekte vaker voor bij hivpositieve mannen die seks hebben met mannen en bij mensen afkomstig uit landen waar hepatitis C frequent aanwezig is. Wereldwijd zijn ongeveer 130-150 miljoen mensen geïnfecteerd, in Nederland zijn er ongeveer 19.200 patiënten met hepatitis C. Er zijn zeven verschillende genetische samenstellingen van het virus bekend, dit noemen we genotypen. Genotype 1 en 3 komen het vaakst voor. Besmetting met het hepatitis C virus geeft in het algemeen weinig tot geen klachten, de ziekte is dus vaak ongemerkt aanwezig. Bij ongeveer een kwart van de mensen kan het eigen immuunsysteem het virus klaren, bij 75% van de mensen blijft het virus echter langer dan 6 maanden in het lichaam, waarna we het een chronische hepatitis C infectie noemen. Als de lever gedurende jaren ontstoken is kan er littekenweefsel (fibrose) en zelfs verschrompeling (cirrose) van de lever ontstaan. Dit moet voorkomen worden, omdat in dit stadium de functie van de lever achteruit kan gaan. In dat geval kunnen er complicaties optreden, zoals decompensatie ofwel ontregeling van de levercirrose met bloed braken, vocht vasthouden en ernstige verwardheid tot gevolg. Daarnaast kan ook een hepatocellulair carcinoom (leverkanker) ontstaan.

Behandeling van hepatitis C

Behandeling heeft het doel het hepatitis C virus permanent te verwijderen uit het lichaam. Hiervoor gebruiken we de maat SVR (Sustained Virological Response, ofwel blijvende virale respons). SVR is gedefinieerd als afwezigheid van het virus in het bloed, 3-6 maanden na het staken van de behandeling. Tot 2012 hadden we maar één type behandeling voor alle genotypen: peginterferon en ribavirine. Deze behandeling duurde lang (24-48 weken), had ernstige bijwerkingen en genezingspercentages waren matig (45-80%). In 2012 was er een doorbraak, de eerste direct-werkende antivirale middelen (DAAs, Direct Acting Antivirals) werden op de Nederlandse markt beschikbaar. Inmiddels zijn er in totaal 3 typen DAAs die direct op het virus aangrijpen: NS3/4A protease remmers, NS5A remmers en NS5B polymerase remmers. Deze middelen kunnen met elkaar gecombineerd worden en met de behandeling behalen we hoge genezingspercentages (>90%) voor patiënten met hepatitis C genotype 1, 2 en 4. Patiënten met genotype 3 blijken lastiger te genezen.

Goedkeuring en registratie van nieuwe geneesmiddelen

Meerdere onderzoeken moeten uitgevoerd worden voordat goedkeuring van een geneesmiddel kan worden verkregen via autoriteiten als Food and Drug Administration (FDA) en European Medicines Agency (EMA). Deze onderzoeken zijn in 3 fasen te verdelen: fase 1, fase 2 en fase 3. De laatste fase wordt met name uitgevoerd voor registratie en toegang tot de markt en worden ook wel registratie studies of registratie trials genoemd. De resultaten van deze studies moeten generaliseerbaar zijn naar de klinische praktijk, zodat artsen goed kunnen inschatten wat de beste therapie voor de individuele patiënt is. De generaliseerbaarheid van de eerste studies met DAAs voor hepatitis C bleek matig te zijn. Daarnaast zouden de nieuwe geneesmiddelen in deze studies vergeleken moeten worden met een relevante andere therapie (bij voorkeur de standaard therapie) zodat artsen meteen weten welke behandeling het beste werkt.

Dit proefschrift heeft als doel de vertaling van hepatitis C registratiestudies naar de klinische praktijk te verbeteren. We hebben hiervoor twee stappen onderzocht: 1) de generaliseerbaarheid van studies bij patiënten met hepatitis C, en 2) de interpretatie van data in de klinische praktijk.

Generaliseerbaarheid van studies naar de klinische prakrijk

Voor **hoofdstuk 2** hebben we een landelijke database opgezet van 467 hepatitis C patiënten met genotype 1, die behandeld zijn met telaprevir of boceprevir gecombineerd met peginterferon en ribavirine. We hebben de gegevens middels statusonderzoek verzameld in 45 ziekenhuizen. Met dit onderzoek toonden we aan dat bijna de helft (47%) van de patiënten die in de Nederlandse praktijk behandeld zijn op basis van de in- en exclusiecriteria uitgesloten zouden worden van de registratiestudies met deze middelen. De uitgesloten patiënten hadden duidelijk meer ernstige bijwerkingen (leidend tot ziekenhuisopname, blijvende beperking of zelfs overlijden) dan de patiënten die wel voldeden aan alle criteria (27% vs. 11%, $p<0.001$). Als we de meest strikte in- en exclusiecriteria hanteerden was ook de effectiviteit van behandeling lager in de groep met uitgesloten patiënten. De patiënten die wel voldeden aan alle in- en exclusiecriteria van de registratiestudies hadden vergelijkbare resultaten met de studiepatiënten. Resultaten van registratiestudies blijken dus alleen generaliseerbaar naar patiënten die voldoen aan alle criteria. We hebben ook gekeken naar de exclusiecriteria die het meeste invloed hadden op effectiviteit en bijwerkingen. Dit bleken zowel een voorgeschiedenis van decompensatie van levercirrose (inclusief bijpassende bloedwaarden zoals trombocytopenie, laag albumine en hoog bilirubine) als bepaalde co-morbiditeit (zoals een hartziekte, maligniteit, anemie en neutropenie) te zijn. Deze criteria zijn vergelijkbaar met belangrijke voorspellers van effectiviteit in huidige grote klinische praktijk cohorten: voorgeschiedenis van (gedecompenseerde) levercirrose, trombocyten, bilirubine, albumine en hemoglobine. Dit suggereert dat de resultaten van onze studie ook relevant zijn in het huidige nieuwe-generatie-

DAA tijdperk. Helaas is het zo dat de volledige set aan in- en exclusiecriteria vaak niet beschikbaar is in de gepubliceerde artikelen. Zelfs het wereldwijde trial register (ClinicalTrials.gov) geeft niet alle criteria weer. Met name criteria gerelateerd aan co-morbiditeit, co-medicatie en bloedwaarden ontbreken. Hierdoor is zorgvuldige beoordeling van generaliseerbaarheid van studies lastig. Patiënten die onvoldoende geïncludeerd worden in trials maar die wel in de klinische praktijk behandeld worden, hebben kans op suboptimale effectiviteit of onverwachte bijwerkingen.

In **hoofdstuk 3** en **hoofdstuk 4** hebben we twee specifieke bijwerkingen (infecties en bloedingen) onderzocht in het cohort van patiënten die behandeld zijn met telaprevir of boceprevir. Deze bijwerkingen zijn al eerder onderzocht bij de combinatietherapie met peginterferon en ribavirine omdat beenmergsuppressie door peginterferon het risico op deze bijwerkingen zou verhogen. In **hoofdstuk 3** vonden we dat 31% van de 467 behandelde patiënten een klinisch relevante infectie ontwikkelde tijdens therapie en 7% van de infecties was ernstig. Deze resultaten kwamen overeen met de infectie percentages bij peginterferon en ribavirine therapie, maar waren hoger dan de infectie percentages in de registratiestudies van telaprevir en boceprevir. We identificeerden de volgende risicofactoren voor infectie: vrouwelijk geslacht, diabetes mellitus en chronisch obstructief longlijden (COPD). Neutropenie was geen risicofactor voor infectie, dus geadviseerde dosisreducties van peginterferon zijn waarschijnlijk niet nodig en kunnen leiden tot verminderde effectiviteit. In **hoofdstuk 4** vonden we dat 22% van de patiënten die behandeld zijn met telaprevir of boceprevir een bloeding ontwikkelden. Echter, het grootste deel (>80%) van de bloedingen was mild qua ernst en behoefde geen behandeling. We vonden 10 ernstige bloedingen: bloed braken (3), ernstige neusbloeding (3), bloedig cerebrovasculair accident (CVA, 2), rectaal bloedverlies en een subduraal hematoom. Het percentage patiënten met een bloeding was hoger dan in onderzoek met peginterferon en ribavirine, mogelijk wordt dit veroorzaakt door telaprevir of boceprevir. Risicofactoren voor een bloeding bleken: ernstige trombocytopenie (bloedplaatjes <50 x10^9/L), vrouwelijk geslacht en levercirrose. Echter, omdat de meerderheid van de bloedingen mild was, is dit probleem vaak niet klinisch relevant.

De ontwikkeling van de nieuwe generatie hepatitis C medicatie ging snel: in de afgelopen jaren zijn er veel studies naar nieuwe middelen gedaan en de huidige behandeling behoeft geen peginterferon meer. Peginterferon had specifieke contra-indicaties en ernstige bijwerkingen, wat invloed gehad kan hebben op de inclusie van patiënten in trials. We verwachtten daarom dat de in- en exclusiecriteria van trials steeds minder streng zouden zijn geworden in de tijd. In **hoofdstuk 5** concludeerden we dat patiënten in de registratiestudies steeds meer lijken op de patiënten uit de klinische praktijk. We onderzochten dit door te berekenen hoeveel patiënten uit een klinisch praktijk cohort (n=177) voldeden aan de in- en exclusiecriteria van registratiestudies van de nieuwe DAA regimes. We vonden een lineaire associatie met de startdatum van de studies: gemiddeld voldeed 75% van de patiënten in 2008 en 89% in 2014 aan de in- en exclusiecriteria. Waarschijnlijk heeft dit geresulteerd in een betere

generaliseerbaarheid. Dit zien we ook in de vergelijkbare resultaten van huidige grote klinische praktijkcohorten en registratiestudies .

Ondanks dit goede nieuws zijn nog niet alle problemen rondom DAAs opgelost. In **hoofdstuk 2** zagen we dat gebruik van co-medicatie de belangrijkste reden voor exclusie uit een registratiestudie was. De achterliggende gedachte is dat DAAs invloed kunnen hebben op geneesmiddeltransporters en enzymen, maar ook beïnvloed kunnen worden door co-medicatie. In **hoofdstuk 6** hebben we het risico op geneesmiddel-geneesmiddel interacties tussen DAAs en co-medicatie onderzocht op basis van de Liverpool database (www.hep-druginteractions.org). We zagen dat 77% van de patiënten co-medicatie gebruikte en dat 60% van de patiënten risico had op een relevante geneesmiddel-geneesmiddel interactie met één van de DAA combinaties. Deze interacties zijn van belang in de klinische praktijk omdat ze kunnen leiden tot verminderde effectiviteit of meer bijwerkingen van zowel de DAA als de co-medicatie. Antidepressiva, protonpompremmers en benzodiazepine derivaten werden het meest gebruikt, maar hebben ook het risico op een interactie met DAAs. De nieuwste generatie DAA regimes (grazoprevir/elbasvir and sofosbuvir/velpatasvir) bleken het laagste risico op interacties te hebben, terwijl de combinatie paritaprevir/ritonavir, ombitasvir en dasabuvir het hoogste risico gaf. Opvallend was dat 23% van de gebruikte co-medicatie (n=60) niet in de database beschikbaar was. Wij voorspelden dat 11 van de 60 medicijnen wel in staat waren een interactie te hebben met een DAA. Onze resultaten kwamen overeen met andere onderzoeken en moedigen aan tot het consulteren van een apotheker bij het starten van hepatitis C therapie.

Interpretatie van data in de klinische praktijk

Uiteindelijk worden alle data, verkregen in fase 1, 2 en 3 onderzoek, zowel gebruikt om goedkeuring van de autoriteiten te krijgen als voor implementatie van de therapie in de klinische praktijk. Dit benadrukt het belang van generaliseerbaarheid van trials. Terwijl de generaliseerbaarheid in de loop van de tijd leek te verbeteren, was volledige interpretatie van data nog niet optimaal. Als arts wil je graag weten of het ene geneesmiddel beter is dan het andere geneesmiddel. Echter, er zijn slechts enkele studies geweest die verschillende DAA regimes direct met elkaar vergeleken en in die gevallen was de vergelijkende arm vaak niet de standaardbehandeling. Toch zijn er manieren om deze data te interpreteren, bijvoorbeeld door het uitvoeren van een netwerk meta-analyse. Een netwerk meta-analyse kan meer dan 2 regimes met elkaar vergelijken door directe en indirecte data met elkaar te combineren. Bayesiaanse statistiek kan dan ingezet worden om de effectiviteit van de behandelingen afzonderlijk te schatten. In **hoofdstuk 7** vonden wij middels deze methode dat sofosbuvir/velpatasvir het beste DAA regime is voor patiënten met hepatitis C genotype 3. We hebben voor hepatitis C genotype 3 gekozen omdat dit genotype het lastigst te genezen is. Daarnaast vonden we dat de toevoeging van ribavirine de effectiviteit significant verbeterde bij alle patiënten (odds ratio 2.6-4.5). Deze bevindingen kunnen makers van richtlijnen helpen om regimes te prioriteren en tevens kunnen ze de arts helpen een keuze voor therapie te maken.

Aangezien de Nederlandse richtlijn, gepubliceerd in 2013, al achterhaald was in 2014, was een update nodig. **Hoofdstuk 8** is de eerste versie van een Nederlands richtsnoer voor de behandeling van hepatitis C, ontwikkeld na de goedkeuring van sofosbuvir. De snelheid van ontwikkelingen in het hepatitis C veld zorgde ervoor dat het niet haalbaar was een volledige richtlijn te schrijven. We voerden een systematisch review uit om alle beschikbare data te achterhalen en formuleerden aanbevelingen op basis van de GRADE methode. Na publicatie ontstond er een samenwerking tussen vijf beroepsverenigingen voor toekomstige versies van het richtsnoer: Nederlandse Internisten Vereniging (NIV), Nederlandse Vereniging van HIV Behandelaren (NVHB), Nederlandse Vereniging van Maag-Darm-Leverartsen (NVMDL), Nederlandse Vereniging voor Hepatologie (NVH) en Nederlandse Vereniging van Ziekenhuis Apothekers (NVZA). Het doel van dit richtsnoer is tweeledig: het bieden van een praktische leidraad aan artsen en het bevorderen van uniforme en hoge kwaliteit behandeling in Nederland (www.hcvrichtsnoer.nl). We bestudeerden aanbevelingen in internationale richtlijnen en pasten deze toe op de Nederlandse situatie. Na belangrijke nieuwe data of goedkeuring van een DAA hebben we de aanbevelingen aangepast.

Conclusies en toekomstperspectief

Ons onderzoek heeft aangetoond dat strenge in- en exclusiecriteria van hepatitis C registratiestudies leidden tot beperkte generaliseerbaarheid van de resultaten. Hierdoor zagen we onverwacht veel ernstige bijwerkingen bij patiënten in de klinische praktijk. We hebben een aantal algemene principes geïdentificeerd om generaliseerbaarheid te verbeteren: 1) alle in- en exclusiecriteria moeten zorgvuldig verantwoord worden, met aandacht voor criteria die gerelateerd zijn aan voorspellers voor effectiviteit of bijwerkingen; 2) de volledige set aan in- en exclusiecriteria van elke trial zou beschikbaar moeten zijn om adequaat de generaliseerbaarheid van een trial te onderzoeken; 3) we hebben subgroepen geïdentificeerd die bestudeerd zouden moeten worden voordat een DAA goedgekeurd wordt, om zo schade in de klinische praktijk te voorkomen (patiënten met gedecompenseerde levercirrose en co-morbiditeit zoals bloedarmoede, maligniteiten of hartziekten); en 4) we toonden aan dat het betrekken van een apotheker van belang is bij het opstarten van therapie, omdat het risico op geneesmiddel-geneesmiddel interacties hoog is en de gepubliceerde artikelen hier onvoldoende aandacht aan schenken.

Gelukkig lijken de trial patiënten steeds meer op de patiënten uit de klinische praktijk. Echter, de introductie van pragmatische trials, studies met een relevante vergelijkende arm, meer toegankelijke 'compassionate use' programma's en inclusie van patiënten die de therapie het hardst nodig hebben in een vroege fase van medicatie ontwikkeling, zouden de generaliseerbaarheid van hepatitis C studies kunnen verbeteren. Verder is een goed patiëntregister van de behandelde patiënten in Nederland nodig om uitkomsten en lange termijn effecten van de medicatie te onderzoeken. Al deze data kunnen gebruikt worden om het werkelijke effect van medicatie in te schatten voor de klinische praktijk en voor richtlijnen. Een voorbeeld hiervan is onze netwerk meta-

analyse. Op dit moment wordt het richtsnoer nog voortdurend aangepast. Zodra de belangrijkste ontwikkelingen achter de rug zijn is het echter tijd voor een Nederlandse richtlijn, gebaseerd op originele data van de studies.

Appendices about the author

Dankwoord

Curriculum Vitae

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RIHS Portfolio

Dankwoord

Lange tijd heb ik gedacht dat het mij nooit zou lukken om te promoveren. Nu ik dit dankwoord mag schrijven is het toch zover: ik mag mijn proefschrift gaan verdedigen. Natuurlijk heb ik dit proefschrift niet alleen geschreven. Juist samenwerking en samen discussiëren maakt onderzoek beter en vooral ook leuker. Graag wil ik iedereen bedanken die hieraan bijgedragen heeft, zowel in wetenschappelijke als persoonlijke zin.

Prof. dr. J.P.H. Drenth, beste Joost, veel dank voor jouw begeleiding in de afgelopen jaren. Ik weet nog hoe ik begon als trial-arts en langzaam de wereld van de farmaceutisch gedreven onderzoeken leerde kennen. Dank voor het vertrouwen wat ik toen van je gekregen heb om dit traject voort te zetten in een promotie traject. Na een lange tijd van dataverzameling kon het schrijven beginnen. Dank voor je geduld en de supersnelle feedback. De 'Floor 7' zal ik niet meer vergeten. Daarnaast mocht ik van jou solliciteren voor de Radboud Da Vinci Challenge, dit heeft me een boost en veel vertrouwen gegeven. Ik vond onze samenwerking erg plezierig en kijk uit naar de volgende fase, de opleiding tot MDL-arts.

Dr. W. Kievit, beste Wietske, zonder jou was dit proefschrift nooit klaar gekomen vrees ik, veel dank voor je hulp. Ik heb genoten van onze wekelijkse gesprekken waarin ik alle vragen aan je kon stellen. We hebben interessante wetenschappelijke discussies gehad, maar ook persoonlijke gesprekken, o.a. in het kader van de Da Vinci Challenge. Jouw invloed is in het hele proefschrift zichtbaar, je altijd kritische blik en vermogen om snel te schakelen is indrukwekkend. Jouw ideeën en enthousiasme over onderzoek werken aanstekelijk. Ik had me geen betere copromotor en begeleider kunnen wensen. Ik hoop dat we nog regelmatig kleine restaurantjes in Nijmegen blijven uitproberen.

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MDL onderzoekersgroep, bedankt voor de fijne tijd. Wat een leuke groep mensen en toekomstige MDL collega's zijn jullie! Kritisch, gezellig en altijd in voor een borrel. Ik ben trots op de ontwikkelingen die we als groep hebben doorgemaakt en op het ontwikkelplan wat we voor onszelf hebben opgezet. De groep collega's en oud-collega's is te groot om iedereen persoonlijk te bedanken, een aantal collega's wil ik speciaal noemen. Hedwig, bijna onze hele onderzoeksperiode hebben we samen gedeeld. Mooi om te zien hoe enthousiast, snel en hard jij kunt werken, én dit kunt combineren met je drukke sociale leven. Ik hoop dat we elkaar in Den Bosch ook vaak buiten het werk blijven zien. Myrte, behalve dat we een kritische onderzoeksblik delen en vaak vergelijkbare feedback geven, delen we ook de hobby beachvolleybal. Ik heb genoten van onze trainingen samen en de tijd dat we elkaar buiten het werk zagen. Jammer dat we niet in hetzelfde ziekenhuis onze vooropleiding doen, maar hopelijk zien we elkaar in de tussentijd nog vaak op het zand of gewoon voor een drankje. Angelique, ondanks dat je er een tijdje uit bent geweest voor je opleiding voelt het alsof we continu hebben samengewerkt. Heerlijk hoe goed jij dingen kunt relativeren! Hopelijk kunnen we over een tijdje nogmaals samenwerken. Karina, jouw open blik en oprechte waardering zijn mooi om te zien, dank voor je positieve feedback als ik weer even in een dipje zat. Lauranne, dank voor alle voorbeelden en tips die je me gegeven hebt. Isabelle, veel tijd hebben we samen op een kamer doorgebracht. Knap hoe jij jouw promotie vormgeeft, dank voor je gezelligheid. Natuurlijk dank ik graag alle andere onderzoekers voor de fijne tijd samen: Titus, Marten, Jos, Mark, Mark, Simon, Loes, Yannick, Anna, Xavier, Dorian, René, Bram, Liyanne, Lisa, Vera, Kelly, Judith, Edgar, Evelyn, Govert, Yasmijn, Tom, Wybrich, en Mieke.

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Judith en Jody, 5 dagen per week van Eindhoven naar Nijmegen rijden is niks aan in je eentje. Dank voor jullie gezelschap en leuke gesprekken in de auto.

Een goede werk-privé balans is mijns inziens essentieel voor goed functioneren op de werkvloer. Ik wil graag alle vrienden en vriendinnen bedanken voor de leuke dingen die we gedaan hebben de afgelopen jaren. Het was een goede afleiding tijdens het onderzoek.

Lieve Yvonne, Maud, Evelien, Laura, Susan, Fien, Eef, Janine, Liesbeth en Karlijn, ik waardeer onze weekendjes en uitjes enorm. We kennen elkaar al zo lang, het is fijn om mezelf te kunnen zijn bij jullie. Lieve Maud, jou wil ik speciaal bedanken dat je mijn paranimf wilt zijn. Je hebt de speciale gave dat iedereen zich fijn voelt bij jou, ik ben blij dat je aan mijn zijde staat vandaag.

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Lieve pap, mam, Kris, Britt en Jans, bedankt voor de interesse en steun de afgelopen jaren. Ondanks dat het af en toe lastig was uit te leggen waar mijn onderzoek over ging of 'hoe de dingen er aan toe gaan' bleven jullie geïnteresseerd. Ik vind het ook heel speciaal dat de afronding van dit proefschrift een familieproject is geworden. Jans, jouw gevoel voor taal is bijzonder, daar moet je iets mee doen, dank voor je feedback op de samenvatting; Britt, veel dank voor het mooie design van het proefschrift en je hulp om InDesign te leren kennen, en Kris bedankt dat je de cover hebt ontworpen op basis van de schilderijen van mam. Ik ben er heel blij mee! En pap en mam, fijn dat jullie altijd klaar staan om mee te denken!

En tot slot, lieve Tom, meer nog dan iedereen heb je alle ups-en-downs van mijn promotietijd en, bijna analoog, van mijn humeur gezien. Ik heb veel respect voor het geduld dat je met me hebt gehad als ik weer eens ging werken in de weekenden of avonden. Zelfs op onze reis had je al het geduld van de wereld als ik een dagje ging werken. Tijdens onze reis hebben we ontdekt dat we elkaar echt aanvullen en dat we op één lijn zitten qua dingen waar we van genieten. Natuur en avontuur is ons ding, en relaxen is niet onze sterkste kant. Ik kijk er naar uit om samen nieuwe avonturen aan te gaan!

Curriculum Vitae

Floor Berden werd geboren op 16 januari 1986 te Horst-America (Limburg). In 2004 behaalde zij haar VWO-diploma aan het Dendron College te Horst. Aansluitend startte zij met de studie geneeskunde aan de Radboud Universiteit Nijmegen. Tijdens de studie ging Floor tweemaal naar Afrika voor de wetenschappelijke stage te Ghana (2008) en het senior coschap te Tanzania (2010). Zij behaalde in 2011 haar artsexamen. Daarna startte zij als ANIOS (arts niet in opleiding tot specialist) Interne Geneeskunde in het Canisius Wilhelmina Ziekenhuis te Nijmegen.

Eind 2012 startte Floor als trial-arts op de afdeling Maag-, Darm-, en Leverziekten van het Radboud universitair medisch centrum. Zij voerde meerdere farmaceutisch gedreven fase 2 en fase 3 trials uit met nieuwe geneesmiddelen voor hepatitis C, primaire biliaire cholangitis, primaire scleroserende cholangitis en inflammatoire darmziekten. Daarnaast behandelde Floor patiënten met hepatitis C op de polikliniek. Floor had de ambitie te promoveren en maakte met prof. dr. Drenth en dr. Kievit een onderzoeksplan, gebaseerd op de ervaringen met de farmaceutische trials en polikliniek. De resultaten hiervan staan beschreven in dit proefschrift. Naast het onderzoek zette Floor zich in voor het ontwikkelplan op de afdeling Maag-, Darm-, en Leverziekten ter bevordering van persoonlijke en professionele ontwikkeling van de arts-onderzoekers. Daarnaast werd zij geselecteerd voor de Radboud Da Vinci Challenge en was zij lid van het Radboudumc Hotspots team. Sinds mei 2017 is Floor in opleiding tot Maag-, Darm-, en Leverarts (opleider. mw. dr. M. van Kouwen). Zij is per 1 mei 2017 gestart met de vooropleiding Interne Geneeskunde in het Jeroen Bosch Ziekenhuis te 's Hertogenbosch (opleider mw. dr. W. Smit).

List of publications

Berden FA, Vuik FE, Drenth JP, Kievit W. The gap between registration trials and real world in hepatitis C is closing. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2017;49(4):454-5.

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RIHS PHD PORTFOLIO

Institute for Health Sciences
Radboudumc

Name PhD candidate: Floor A.C. Berden
Department: Gastroenterology and Hepatology
Graduate School: Radboud Institute for Health Sciences

PhD period: 01-11-2013 – 31-12-2016
Promotor: Prof. dr. J.P.H. Drenth
Copromotor: Dr. W. Kievit

		Year(s)	ECTS
TRAINING ACTIVITIES			
a)	**Courses & Workshops**		
-	Introduction day Radboudumc	2013	0.5
-	BROK course	2013	1.75
-	Ultrasonography of the liver course	2013	0.4
-	Fibroscan course	2013	0.2
-	Young Investigators Meeting, United European Gastroenterology	2014	1.75
-	Biometrics Course	2014	3.75
-	Scientific Integrity Course	2015	1.0
-	Summerschool, United European Gastroenterology	2016	1.75
b)	**Seminars & lectures**		
-	IGMD PhD retreat: poster presentation	2013	0.75
-	PhD retreat department of gastroenterology and hepatology	2015, 2016	1.5
c)	**Symposia & congresses**		
-	Nederlandse Vereniging voor Gastroenterology (NVGE) congress: attendence and one oral presentation	2013, 2016	0.75
-	European Association for the Study of the Liver (EASL) congress: attendence	2013, 2014, 2015 2015, 2016	3.0 3.0
-	United European Gastroenterology Week: attendance and 3 poster presentations	2013	0.5
-	Dr. Falk symposium: attendence	2014, 2016	0.25
-	National Hepatitis Day: attendence	2014	0.5
-	National Hepatitis Symposium: attendance and oral presentation (debate)		

Radboud University Radboudumc university medical center

d)	**Other**		
	- Weekly journal club and research meeting of department	2013-2016	12.0
	- Two-monthly regional education gastroenterology and hepatology	2013-2016	2.0
	- Radboud Da Vinci Challenge	2016	4.25
	- Development plan PhD-students Gastroenterology and Hepatology: development and maintenance; focus on feedback training, peer-to-peer consultation (intervisie) and writing-weekend	2014-2016	5.0
	- Member of hepatitis C guidance committee	2015-2016	1.0
	- Member of Radboud Hotspots team	2014-2016	1.0
TEACHING ACTIVITIES			
e)	**Lecturing**		
	- Multidisciplinary Symposium for hemophilia	2013	0.1
	- Viral Hepatitis Prevention Board Meeting	2013	0.1
	- Nursing congress, presentation hepatic diseases and workshop non-alcoholic fatty liver disease	2014	0.2
	- Diner Pensant, regional education Utrecht	2014	0.1
	- National hepatitis day for patients	2014	0.1
	- Presentation in Gelderse Vallei, Ede	2015	0.1
	- Presentation at RAMS summerschool Radboud University	2015	0.1
	- Education for pharmacologists	2016	0.1
f)	**Supervision of internships / other**		
	- Supervision of two medical students (3 months each)	2014-2015 2015-2016	2.0 3.0
	- Supervision of two biomedical students (bachelor student 3 months and master student 6 months)	2016	1.0
	- Supervision of one medical student assistant (4 months)		
TOTAL			**53.5**